Development of New Late-Stage Labeling Methods with Labeled Carbon for Stabilized Carboxylic Acids and α-Amino Acids

by

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ABSTRACT

Isotope labeling of small molecules is a crucial tool for drug discovery and understanding biochemical processes. Expanding the toolbox of a radiochemist with methods that allow late-stage labeling is therefore important. Of the methods that have been developed, the use of isotopically labeled $*CO_2$ (* = 14, 13, 11) precursor is among the most useful platforms for installing isotopically-labeled carbon into the molecule. While this area has undergone a tremendous amount of development in recent years, the preparation of *C-labeled molecules (* = 14, 13, 11) remains difficult and time consuming, with established methods involving label incorporation at an early stage of synthesis. This thesis describes the development of new methods to label stabilized carboxylic acids and α-amino acids with $*CO_2$ (* = 14, 13, 11). It describes reversible decarboxylation/carboxylation reactions by stabilized carboxylic acids and their derivatives that prepare isotopically-labeled carboxylic acids. Through careful pilot studies, reaction optimization, and mechanistic probes, the achiral aryl aldehyde catalyzed isotopic carboxylate exchange of native α-amino acids with $*CO_2$ (* = 14, 13, 11) was discovered. This reaction likely proceeds via the trapping of $*CO_2$ by imine-carboxylate intermediates to generate iminomalonates that are prone to monodecarboxylation. Subsequent work revealed that a chiral aldehyde receptor can mediate the resolution/carboxylate exchange of native α-amino acids with $*CO_2$.

PREFACE

The research conducted for this thesis was performed in collaboration with Prof. Rylan Lundgren.

The synthesis of isotopically-labeled carboxylic acids via direct reversible decarboxylation of stable organic acids in a polar aprotic solvent, described in Chapter 2, was published by Duanyang Kong, Patrick J. Moon, Erica KJ Lui, Odey Bsharat, and Rylan J. Lundgren. *Science* **2020**, 369, 557–561. The reaction discovery was done by Patrick Moon, Duanyang Kong assisted with the optimization, scope, and mechanistic studies, Erica Liu assisted with the scope, and I helped with the scope of the reaction, functionalization, and derivatization of the product.

The synthesis of isotopically-labeled racemic α-amino acids, using achiral aldehyde catalysis in conjunction with isotopically-labeled CO_2, described in Chapter 3 was published by Odey Bsharat, Michael G. J. Doyle, Maxime Munch, Braeden A. Mair, Christopher J. C. Cooze, Volker Derdau, Armin Bauer, Duanyang Kong, Benjamin H. Rotstein, and Rylan J. Lundgren *Nat. Chem* **2022**, *14*, 1367-1374. The reaction discovery, optimization, and selected scope examples are my original work. Michael G. J. Doyle assisted with most of the scope and some mechanistic studies. Christopher J. C. Cooze assisted with selected scope examples and some mechanistic studies. Duanyang Kong assisted with selected scope examples. The work conducted in the thesis to prepare ^{11}C- α-amino acids was a collaboration with Maxime Munch and Braeden A. Mair at uOttawa. They did the scope examples to prepare ^{11}C- α-amino acids. The work conducted in the thesis to prepare ^{14}C-α-amino acids was a collaboration with Volker Derdau and Armin Baueat at Sanofi. They did the scope examples to prepare ^{14}C- α-amino acids.

The synthesis of enantioenriched isotopically-labeled α-amino acids, using chiral aldehyde auxiliary in conjunction with isotopically-labeled CO_2, described in Chapter 4, currently is being developed. I conducted the experiments that constitute the findings in Chapter 4.

DEDICATION

Dedicated to my beloved parents (Falah Bsharat and Nimah Bsharat), my wife (Lana Zubaydi) whose continuous prayers and inspiration led to the accomplishment of this study, as well as to my amazing kids (Adam and Yara).

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First and foremost, I would like to thank Prof. Rylan Lundgren for his great guidance, help, and support over the last five years of this journey. Thank you for having confidence in my ability, through the good times and the bad, and thank you for pushing and supporting me to be the best version of myself.

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I'd like to thank the members of the Lungren group, both past and present, for making my PhD experience a great one. Specific thanks go out to Patrick Moon, Raphael Dada, Duanyang Kong and Christopher J. C. Cooze for your guidance as I started my research in the lab, your feedback, and advice; it is something I still think about to this day. To the members who joined after I did, thank you for challenging me to be a better chemist while always questioning the rationale behind decisions I make. Specific thanks to Wesley McNutt, I thank you for your help and support all the time. To Mike Doyle, thank you for always taking time to edit documents such as scholarship applications and this thesis. To the rest of the Lungren group, Qiqige Qiqige, Alex Gabbey, Markus Shoetz, Anis Fahandej-Sadi, Bohdan Sosunovych, Lars Ross, Yang Ou, Yiyang Ma, Hima Manchacheri Kozhummal, Kai Xu and Sandip Das, it was great working alongside of you for the time we all shared in the lab.

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Finally, I want to acknowledge and thank Dr. Anna Jordan for her great help, support, and guidance in editing this thesis as well as my English teacher, Dr. Andrea Pietrzyk, for her support and editing my thesis and being helpful all the time.

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LIST OF ABBREVIATION AND SYMBOLS USED

aq.	aqueous
°C	degrees Celsius
ADME	absorption, distribution, metabolism, and excretion
Ar	generic aryl moiety
Ac	acetyl
Atm	atmosphere
Bn	benzyl
Bu	butyl
Boc	*tert*-butyloxycarbonyl
B(pin)	pinacol boronic ester
cat.	catalytic stoichiometry
Cy	cyclohexyl
COD	cyclooctadiene
CSA	camphor-10-sulfonic acid
δ	chemical shift
DMPK	drug metabolism and pharmacokinetic
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DCM	dichloromethane
DMA	*N*,*N*-dimethylacetamide
DMF	*N*,*N*-dimethylformamide
DMAP	dimethylaminopyridine
DMSO	dimethyl sulfoxide
DPPF	1,1'-bis(diphenylphosphino)ferrocene
DM	4,4′-dimethoxytrityl
DME	1,2-dimethoxyethane
equiv.	equivalents
Et	ethyl

FDA	Food and Drug Administration
[^{18}F] FDG	2-deoxy-2-[^{18}F] fluoroglucose
HRMS	high resolution mass-spectrometry
hADME	human absorption, distribution, metabolism, and excretion
HIE	hydrogen isotope exchange
HB(pin)	4,4,5,5-tetramethyl-1,3,2-dioxaborolane
KIE	kinetic isotope effect
MS	mass spectrometry
*i*Pr	iso-propyl
LED	light-emitting diode
Liq	liquid
Me	methyl
MeCN	acetonitrile
Et_3N	triethylamine
NMR	nuclear magnetic resonance
NFSI	*N*-fluorobenzenesulfonimide
NMO	*N*-methylmorpholine-N-oxide
OAc	acetate
OTf	triflate
Ph	phenyl
PET	Positron Emission Tomography
PiB	Pittsburgh compound B
QWBA	quantitative whole-body autoradiography
rac	racemic
R	generic group
rt	room temperature
RSNOs	S-nitrosothiols
RCY	radiochemical yield
RCC	radiochemical conversion
SIL	stable isotope labeled

SET	single electron transfer
*t*Bu	*tert*-butyl
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMEDA	tetramethylethylenediamine
TBAF	tetra-n-butylammonium fluoride
TFA	trifluoroacetic acid
TPAP	tetrapropylammonium perruthenate
TE	trapping efficiency
UV	ultraviolet
UBT	urea breath test

CHAPTER 1

Introduction

1.1 Isotopic Labeling in Drug Discovery

There is increased pressure on the chemical industry and allied fields to produce compounds with less waste from non-toxic starting materials, while simultaneously reducing manufacturing costs. Synthetic methodology and homogeneous catalysis can offer solutions to these challenges.[1] Synthetic chemistry is the primary method used to produce and identify new medicines.[2] The development of new synthetic methods impacts on the pharmaceutical industry in two different ways, first by increasing overall efficiency and second by enabling access to novel chemical matter.[3-5] Discovering, developing, and designing new medical drugs is a time-consuming and complex. It requires between 12 to 15 years to take a drug from project initiation to market, with costs of around $2.6 billion per new entity.[6-7]

The drug development process occurs over several steps. The first step involves identification of the medical need and the attempt to find the proper medication. Next comes the drug discovery part, which involves identifying the interaction between the drug candidate and the disease marker. After that is the preclinical phase, which involves in vitro and in vivo studies for early administration, distribution, metabolism, excretion (ADME) studies, and toxicology screening. Then comes testing the safety and potential risks, dose range, and pharmacodynamics properties to determine the efficacy of the drug in a small to medium group of patients (Phase I to Phase II). Phase III involves trying the drug candidate in a large group of patients. After the drug candidate is successful and approved by authorities, it can be launched to the market. Multi-disciplinary teams work together over many years to develop a new drug to treat a particular disease.[8] Unfortunately, less than 10 percent of potential drugs currently reach the market. These low success rates may be due to a weak understanding of the biological and rarely unexpected adverse effects of the drug candidate, which is usually

observed in Phase II clinical trials. Improved methods are required to produce essential human pharmacodynamic and pharmacokinetic data in early drug discovery.

During various stages of drug discovery and development, several in vitro and in vivo studies are carried out to give a better understanding of ADME properties of the drug candidate. The Food and Drug Administration (FDA)[9] recommends Phase 0 clinical trials to provide an alternative early drug development strategy by micro-dosing healthy volunteers with pharmaceuticals that are radiolabeled. The drug candidates can be synthesized with long-lived radionuclides (^{3}H or ^{14}C) and/or short-lived radionuclides (^{11}C or ^{18}F). Labeling the drug candidate with these radioisotopes enables the follow-up of the in vivo fate of the drugs, as these radioisotopes serve as a traceless.[10] The synthesis of radiolabeled compounds is a complex, time-consuming process and usually needs multistep synthesis, while taking into account that the labeling position must be introduced into a metabolically stable position. Therefore, there is a great demand and need for introducing and developing efficient methods to introduce isotopic labeling for the drug candidate to help with the drug discovery process.

1.2 Isotopically-Labeled Compounds and Their Importance

1.2.1 Classifications of Isotopically Labeled Compounds

Isotopes are members of a family of an element that have the same number of protons but different numbers of neutrons in the nucleus. Isotopes are divided into two classes, stable isotopes (for example ^{2}H, ^{13}C, and ^{15}N), and radionuclide isotopes (for example ^{3}H, ^{11}C, ^{14}C, and ^{18}F). Stable isotopes are naturally occurring isotopes that differ from their parent atom (the most abundant form of the element) by a difference of one or more neutrons in the nucleus. Stable isotope-labeled (SIL) compounds are molecules that have at least one atom substituted with a stable isotope.

Specialized isotope chemistry research teams exist in many big pharmaceutical companies. The main goal of these units is to provide labeled compounds for metabolism-based studies. Alternatively, many custom research organizations serve the pharmaceutical sector by focussing solely on the preparation of isotopically labeled

compounds. In vitro and in vivo studies are performed to get a better understanding of the absorption, distribution, metabolism, and excretion properties of the drug candidate during the various stages of drug development.[10] To facilitate these studies, drug candidates can be synthesized with stable nuclides (^{2}H, ^{13}C, or ^{15}N), long-lived radionuclides (^{3}H or ^{14}C), and/or short-lived radionuclides (^{11}C or ^{18}F) (**Fig. 1–1**).

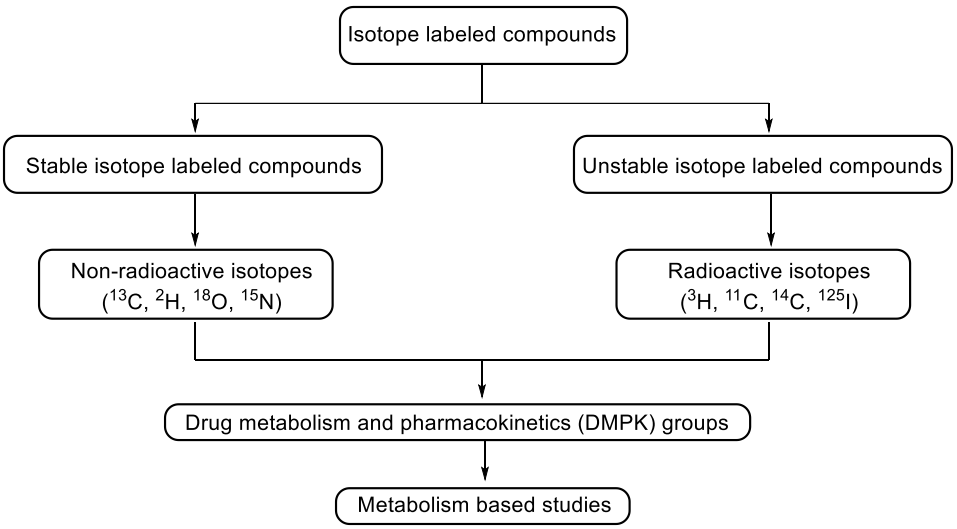


Fig. 1–1. Classifications of isotopically-labeled compounds.

1.2.2 The Uses of Isotopically Labeled Compounds

SIL compounds play an important role in understanding the behaviour of the drug, i.e., pharmacodynamics and pharmacokinetics, mechanism of toxicity and adverse effects, and its bioavailability.[11] SIL compounds provide a unique mass from the unlabeled compound, which is why they can be used in mass spectrometry (MS) for the absolute quantification of the parent compound and relative quantification of metabolites from biological samples.[12]

The most used stable isotopes in drug molecules are ^{2}H, ^{13}C, and ^{15}N. The selection of the stable isotope to incorporate in the drug molecules depends on several factors, including cost, commercial availability, and the synthetic methods available. Deuterium has a large isotope effect, which can alter the drug molecule properties significantly in comparison to ^{13}C and ^{15}N. Deuterium also is prone to wash out if it is on an exchangeable position and is prone to metabolic shifting.[13]

SIL compounds can be used as clinical agents. For example, Deutetrabenazine (**Fig. 1–2**) is an approved drug for the treatment of both tardive dyskinesia and Huntington's disease. Tardive dyskinesia causes a range of repetitive muscle movements in the face, neck, arms, and legs, while Huntington's disease causes the progressive breakdown of nerve cells in the brain. This drug is an improved treatment over tetrabenazine. When the hydrogen is replaced by deuterium atoms, the drug has better metabolic stability.[14-15] Another example of a stable isotope drug as a clinical agent is the [^{13}C]urea breath test,[16] which is utilized in one of the most important non-invasive methods for detecting a Helicobacter pylori infection (**Fig. 1–2**).

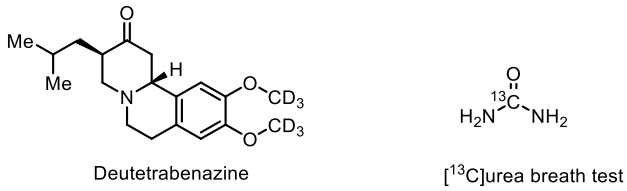


Fig. 1–2. Deutetrabenazine and [^{13}C]urea breath test.

The second class of isotopes is the radioactive isotopes. A radioactive isotope is an isotope that has excess nuclear energy, making it unstable. Long-lived radionuclides such as ^{3}H, and ^{14}C are used widely throughout pharmaceutical research and development.[17] Tritium-labeled compounds are used by drug metabolism and pharmacokinetic (DMPK) groups to collect early metabolism data to assess covalent binding of reactive metabolites;[18] they also can be used for quantitative whole-body autoradiography (QWBA).[19] ^{14}C-labeled compounds are used in Phase III of the drug development process for quantitative whole-body autoradiography and mass balance studies, human absorption, distribution, metabolism, and excretion studies, and for environmental fate studies.[19-21] Tritium-labeled compounds are synthesized more easily and rapidly than ^{14}C-labeled compounds, but they have a greater potential for loss of the label by chemical and biological processes.[21]

On the other hand, the main use of short-lived radionuclides such as ^{11}C and ^{18}F is as radiotracers in Positron Emission Tomography (PET) techniques. PET is a non-invasive nuclear imaging technique used in clinical diagnosis, drug development, and biomedical research.[22] The major applications of PET in drug development process

include the microdosing approach, in which a study for in vivo distribution of a labeled drug is performed; target occupancy studies, in which the in vivo drug-target interaction is studied; and biomarker studies, in which an imaging biomarker can be used to follow the development of the disease and to monitor the outcome of treatment.[23]

Carbon-14 has a half-life of 5730 years. This long-lived radioisotope emits low-energy β-particle radiation that is utilized commonly as a traceless tag for organic molecules to study their fate.[24] Carbon-14 radiolabeling plays an important role in drug development and the agrochemical industry in many aspects: in the pharmaceutical development discipline to understand drug metabolism, disposition, and pharmacokinetics of pharmaceuticals;[25-26] in the animal health drug development discipline to determine the metabolism, disposition, and pharmacokinetics of veterinary drugs;[27] in crop science to understand the plant metabolism of agrochemicals and pesticides;[28] and in environmental fate studies to perform soil dissipation studies and assess potential environmental impacts associated with human and animal health product excretions that might enter the aquatic and terrestrial environment.[29-30]

Carbon-11 has a half-life of 20.3 min. Carbon-11 labels are used as a PET radionuclide to visualize and measure changes in metabolic processes, to trace a radiolabeled drug in living tissues, and in other physiological activities, including oncology and neurology.[31] Examples of using ^{11}C-labeled compounds include vinpocetine, which is used as a neuroprotective drug in the treatment of ischemic stroke; using the labeled version of the drug [^{11}C]vinpocetine helped to confirm the possibility of a central mechanism of action.[31,32] ^{11}C-labeled compounds also can be used as biomarkers for pathophysiology, and PET Imaging amyloid plaques with C-Pittsburgh compound-B [^{11}C]PIB) have the potential to facilitate quantification of the dissolution of amyloid plaques in Alzheimer's disease after effective treatment.[33-34] Another example is the use of [^{11}C]choline for imaging various tumors, including prostate cancer, lung cancer, and brain tumor.[35–40] The negligible uptake of the tracer in the normal brain allowed good delineation of brain tumor contours and diagnosis of tumor recurrence.[35-37]

1.3 Classical Methods for Isotopic Labeling Via Functional Group Transformations

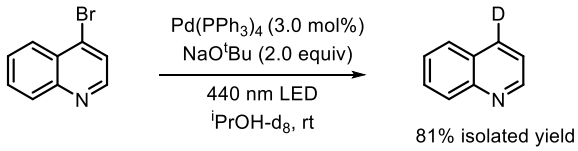
1.3.1 Deuterium or Tritium Labeling of Organic Compounds

The incorporation of deuterium or tritium into an organic molecule can be achieved through two main ways, by functional group transformation or by direct hydrogen isotope exchange (HIE). The most common deuterium sources include D_2O, D_2, $CDCl_3$, MeOD, AcOD, EtOD, DMSO-d_6, MeOH-d_4, Acetone-d_6, CD_3CN, C_6D_6, CD_3OH, ^{i}PrOD, DCO_2D, and ^{i}PrOH-d_8.[41]

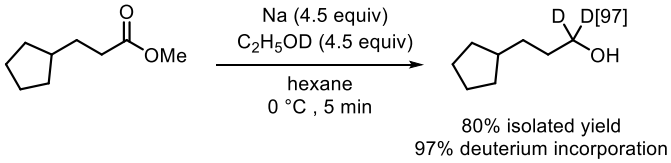
Although HIE is the most common method for these transformations, H-2 and H-3 labeling by functional group transformation can offer greater than a 99% incorporation as well as complete selectivity. Incorporation by functional group transformation includes reductive deuteration, such as a photoinduced palladium-catalyzed deuterodehalogenation of inactivated aryl and alkyl bromides,[42] reductive deuteration of carbonyl group,[43] and olefins[44] (**Fig. 1–3**). Boron catalyzed hydrodeuteration of olefin (**Fig. 1–3C**) proceeds via abstraction of a deuteride from the cyclohexadiene (**1-A**) by the borane Lewis acid catalyst to form ion pair intermediate $\mathbf{I}^{+}[DB(C_6F_5)_3]^{-}$ (**1-B**), Then protonation of styrene (**1-C**) by Wheland complex I^{+} leads to II+$[DB(C_6F_5)_3]^{-}$ (**1-D)**. Finally, deuteride transfer provides the final monodeuterated product (**1-E)** and returns the $B(C_6F_5)_3$ catalyst as shown in **Fig 1-3C**.

Also, it includes different transformations, such as formation of trideuteromethylation[45] and deuterated aldehydes[46] (**Fig. 1–4**).[41]

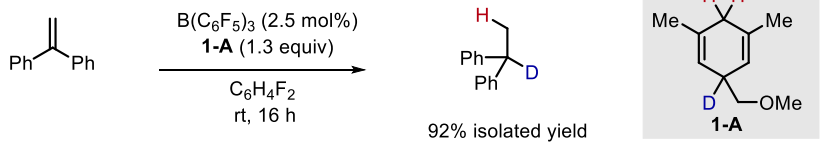
a. Visible light-mediated, palladium-catalyzed deuterodebromination



b. Reductive deuteration of aliphatic esters by sodium dispersions



c. Boron-catalyzed hydrodeuteration



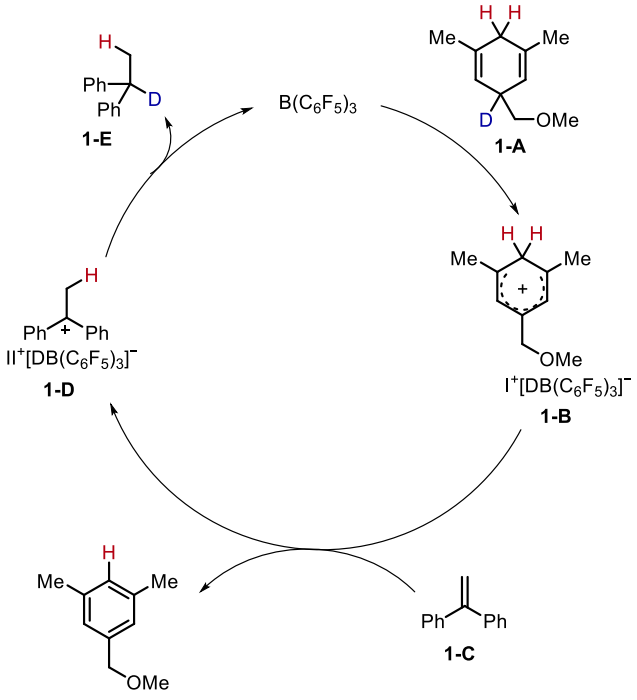


Fig. 1–3. Labeling with ^{2}H and ^{3}H by reductive deuteration.

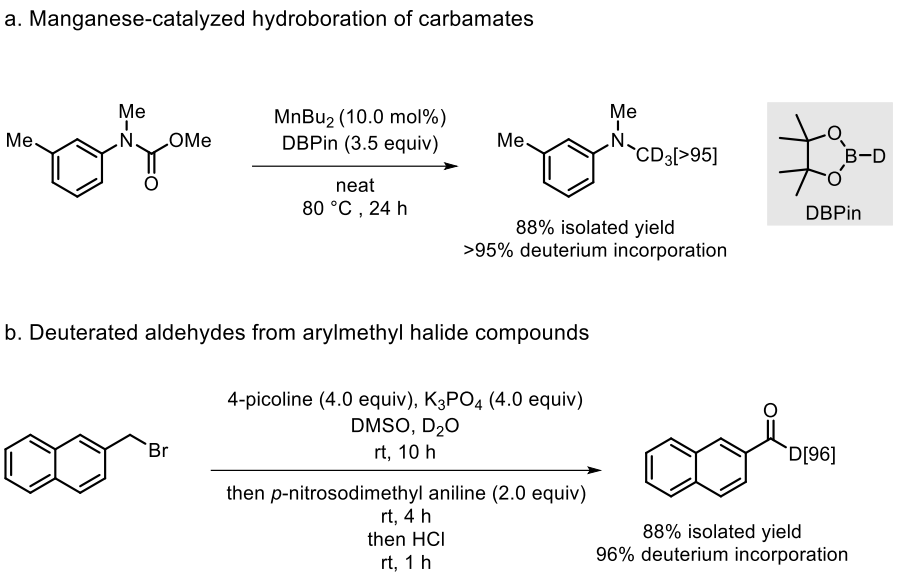


Fig. 1–4. Labeling with ^{2}H and ^{3}H via formation of trideuteromethylation and deuterated aldehydes.

1.3.2 ^{18}F-Labeling of Organic Compounds

Fluorine-18 has a relatively long half-life ($t_{1/2}$ = 110 min). It is the most common radioactive isotope for PET studies due to its physical and nuclear properties.[47-48] Additionally, one of the most common radiotracers in the clinic is 2-deoxy-2-[^{18}F]fluoro-D-glucose PET radiotracer (2-[^{18}F]FDG).[49,50]

Typical challenges associated with fluorine-18 radiochemistry include the use of sub-stoichiometric amounts of ^{18}F, relative to the precursor and other reagents, and the limited availability of parent ^{18}F sources of suitable reactivity ([^{18}F]F$^-$ and [^{18}F]F$_2$). In general, the incorporation of ^{18}F into an organic molecule can be achieved by using nucleophilic reagents, such as K^{18}F/Kryptofix[2.2.2] (**Fig. 1–5a**) and tetrabutylammonium [^{18}F]fluoride (TBA[^{18}F]F) (**Fig. 1–5b)**,[51] and by using electrophilic reagents, such as [^{18}F]-*N*-fluorobenzenesulfonimide ([^{18}F]NFSI) or [^{18}F] selectfluor bis(triflate) (**Fig. 1–5c**).[52-54]

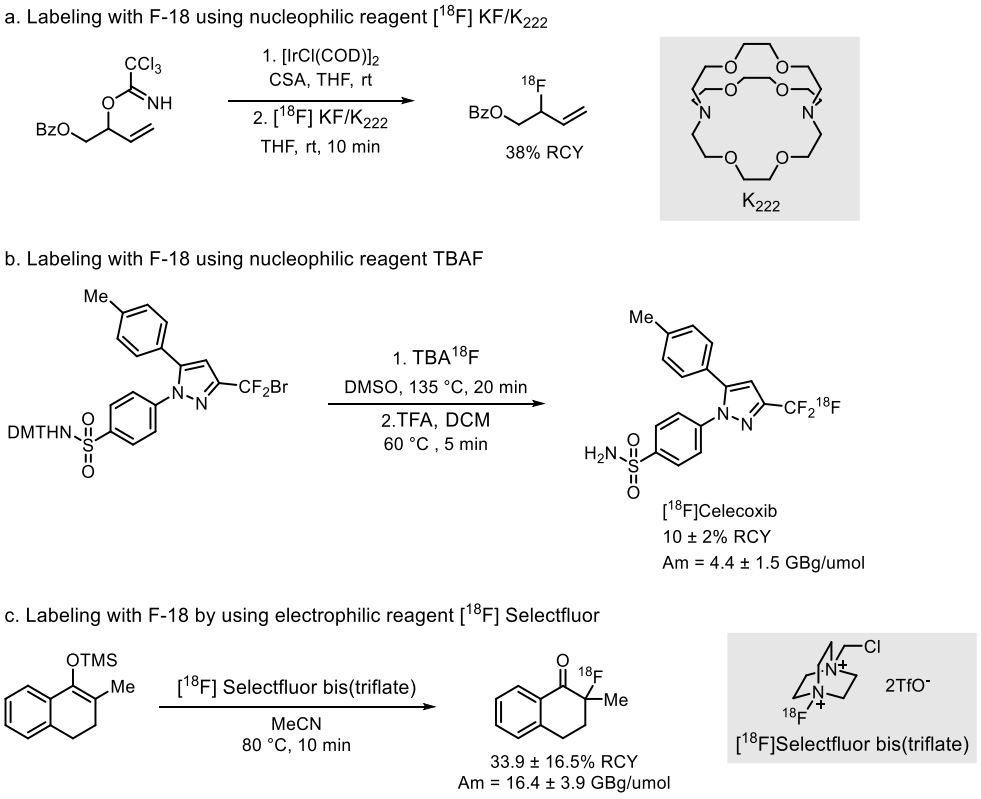


Fig. 1–5. Labeling with F-18 by using nucleophilic and electrophilic reagents.

The incorporation of ^{18}F into an organic molecule to make CF_2 [^{18}F]-labeled molecules can be achieved via halex exchange processes (**Fig. 1–6a**),[55-61] fluorodecarboxylation of arylCF_2COOH (**Fig.1–6b**),[62-64] in situ generated of [^{18}F]$CuCF_3$ (**Fig. 1–6c**),[65] and by generating isolable precursor bis(trifluoromethyl)Au(III) complexes (**Fig. 1–6d**).[66]

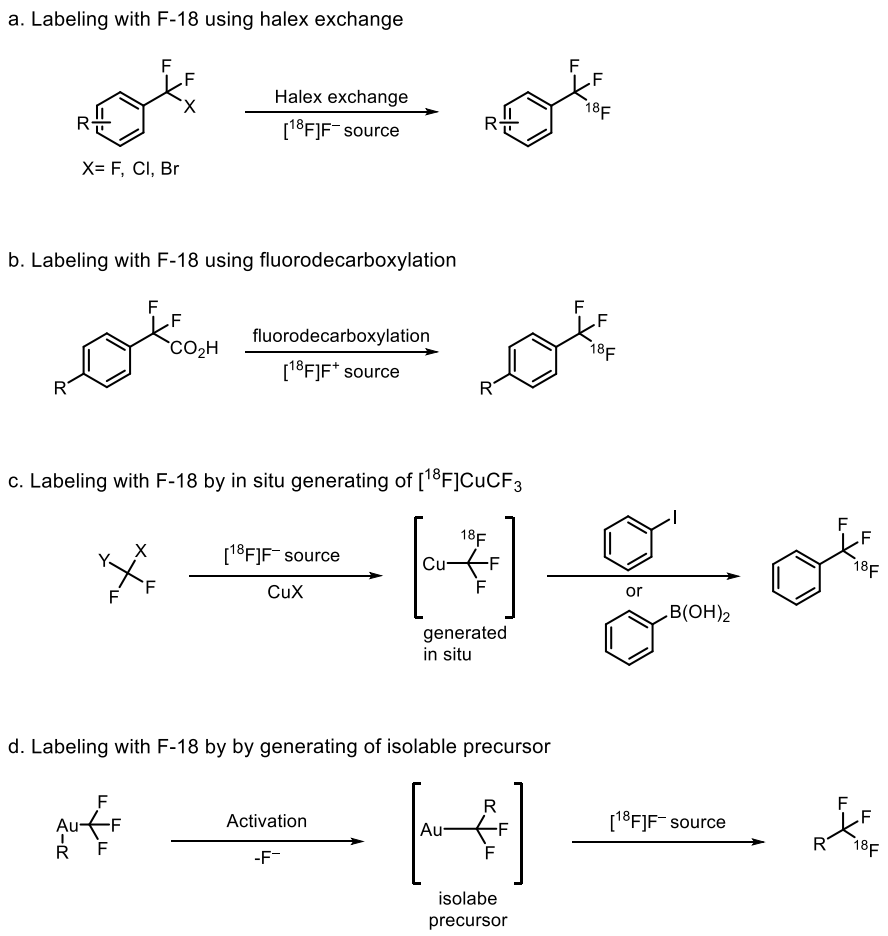


Fig. 1–6. Methods to make CF_2 [^{18}F]-labeled molecules.

The incorporation of ^{18}F into aromatic compounds can be achieved by fluorodemetalation of preformed palladium or nickel arene complexes from the requisite aryl halides or boronic acids[67-68] and by copper-mediated cross-coupling of preformed or in situ–generated aryliodoniums,[69-70] or aryl boronic acids[71] (**Fig. 1–7a**). Also, it can be done by direct arene C–H fluorination with $^{18}F^-$ via organic photoredox catalysis (**Fig. 1–7b**).[72]

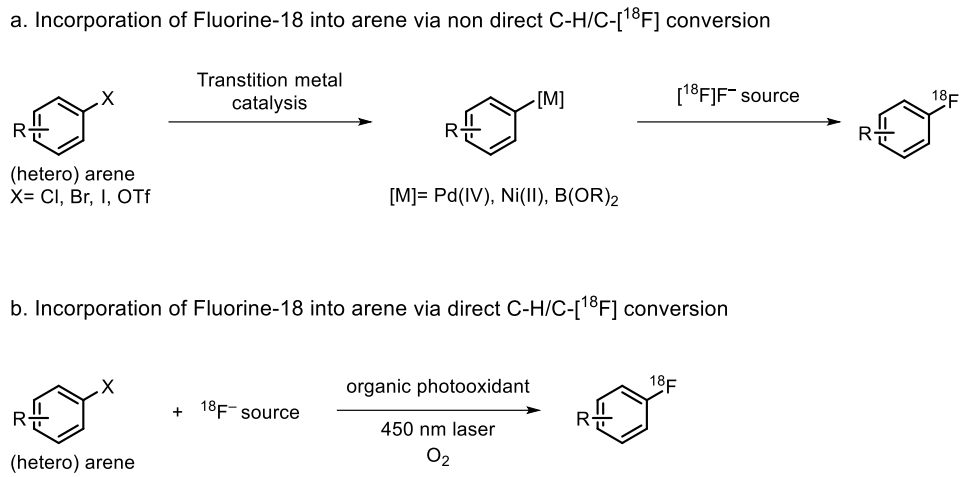


Fig. 1–7. Methods for incorporation of ^{18}F into arene.

1.3.3 ^{15}N-Labeling of Organic Compounds

Natural nitrogen consists of two stable isotopes: the vast majority (99.6%) of naturally occurring nitrogen is ^{14}N, with the remainder being ^{15}N. The incorpotion of ^{15}N into organic molecules can be done by using ^{15}N-labeled building blocks. An example of these is building ^{15}N-labeled ammonium, nitrite, and nitrate salts.[73] A second building block is the [^{15}N]NO building block, an example of which is [^{15}N]NO reacting with diethylamine or diethylammonium salts to produce ^{15}N-labeled diazeniumdiolates.[74] A third building block is the [^{15}N]NO_2^- building block, an example of which is ^{15}N-labeled RSNOs, such as ^{15}N-labeled S-nitrosocysteine (S^{15}NC) and S-nitrosoglutathione (GS^{15}NO),[75] which are obtained by reacting [^{15}N]NO_2^- in acidic medium with the corresponding thiols (**Fig. 1–8**). In general, ^{15}N-labeled amino acids are used to elucidate their metabolism, ^{15}N-labeled S-nitrosocysteine (S^{15}NC) can operate S-transnitrosation with the thiol group of cysteine residues of proteins, and ^{15}N labeled *S*-nitrosoglutathione (GS^{15}NO), one of the most abundant low-molecular-mass RSNOs in cells, has been labeled with ^{15}N to study *S*-transnitrosation.[73]

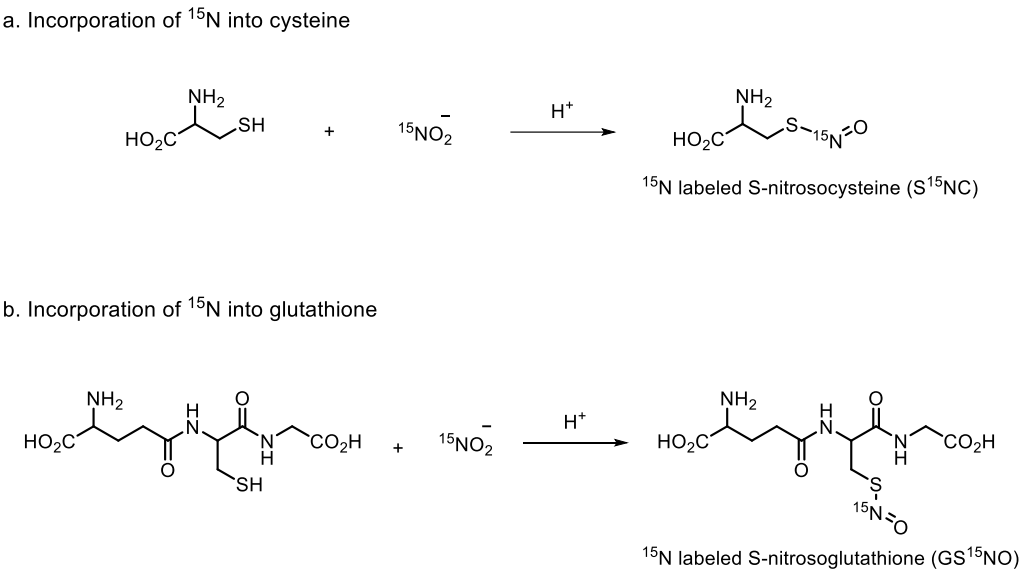


Fig. 1–8. Methods for incorporation of ^{15}N into cysteine and glutathione.

1.3.4 *C-Labeling of Organic Compounds [*C = ^{14}C, ^{13}C, or ^{11}C]

1.3.4.1 $^{13/14}$C-Labeling of Organic Compounds

Carbon has 15 isotopes, from ^{8}C to ^{22}C, of which ^{12}C and ^{13}C are stable isotopes. The longest-lived radioisotope is ^{14}C, which has a half-life of 5730 years. The incorporation of ^{13}C and ^{14}C into the organic molecule via functional group transformation can be done by using isotopically-labeled building blocks.

Isotopically-labeled building blocks include metal [*C]cyanides (M = Na, K, Zn, Cu),[76] which are useful building blocks for introducing *****C [*****C = ^{14}C, ^{13}C] into an organic molecule (**Fig. 1–9a**). While these solids are easier to handle, many M*CN are hygroscopic and all are toxic; therefore, care must be taken when handling them. The second building block is the [$^{14}C_2$]acetylenes building block. For example, Elmore et al. reported the synthesis of triphenylsilyl [$^{14}C_2$]acetylene for the use in Sonogashira reaction (**Fig. 1–9b**).[77] The third building block is the [^{14}C]cyanamide building block. For example, Murthy et al. reported the use of [^{14}C]H_2NCN to incorporate a C-14 label on the position of a pyrimidine ring, as shown in (**Fig. 1–9c**).[78] The fourth building block is the [^{14}C]methyl iodide, which is one of the most frequently used building blocks, being the source of both electrophilic and nucleophilic one [*C] carbon

synthons. An example of the use of [^{14}C]methyl iodide is the synthesis of [*S*-methyl-^{14}C]methionine (**Fig. 1–9d**).[79]

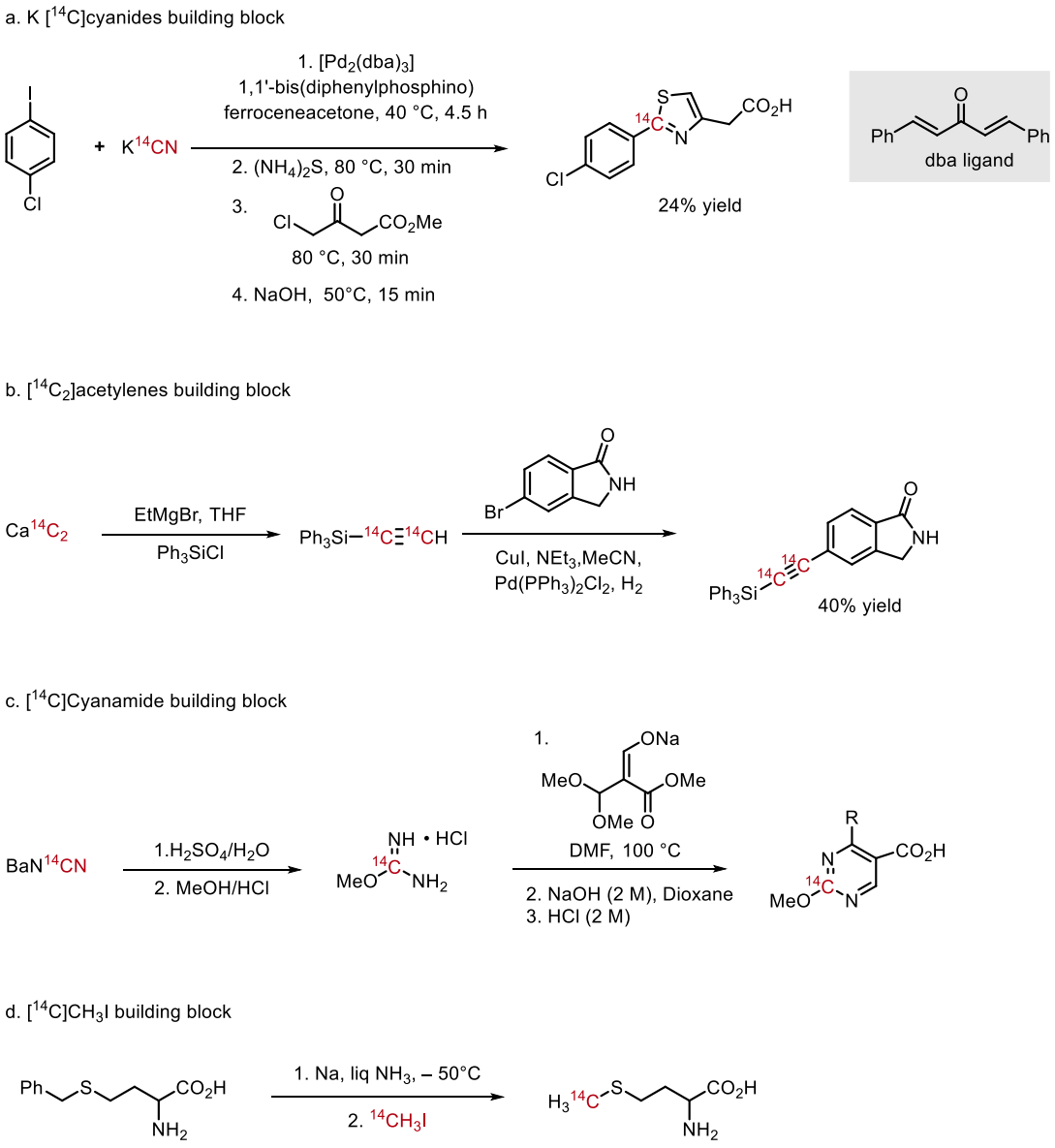


Fig. 1–9. [13/14] C-Labeling of organic compounds with labeled building blocks: a. K[^{14}C]CN building block, b. [$^{14}C_2$]acetylenes building block, c. [^{14}C]cyanamide building block. d. [^{14}C]CH_3I building block.

Isotopically-labeled building blocks also include CO; an example of that is the synthesis of ^{14}C-carboxylic acid from the more complex substrate *o*-chloroaryl iodide to provide the product in 87% chemical yield and 32% overall radiochemical yield from barium [^{14}C]carbonate. The product is a key intermediate in the preparation of a series

of labeled gonadotropin-releasing hormone agonist (GnRH agonist) of the amide type (**Fig. 1–10a**);[80] the [^{14}C]HCOOH building block, an example of which is the synthesis of 3-deaza[8-14C] adenosine derivative, which is a key intermediate in the synthesis of 3-deaza[8-14C] adenosine (**Fig. 1–10b**);[81] the [^{14}C]HCHO building block, an example of which is used for the synthesis of the corresponding alkenylamine provided *N*-benzyl[2,6-^{14}C]piperidin-4-ol, which served as key intermediate to the CCR5 receptor antagonist [^{14}C]SCH 351125 (**Fig. 1–10c**);[82] the [^{14}C]CH_3NO_2 building block, an example of which is used for the synthesis of [2-^{13}C]ketoglutaric acid, which was used for investigations in the biosynthesis of cephalosporins (**Fig. 1–10d**).[83]

In addition to all the previously mentioned building blocks is the isotopically-labeled carbon dioxide building block, *CO_2 [*C = ^{14}C, or ^{13}C], which can be introduced via carboxylation of organometallic compounds and coupling with organomagnesium or organolithium reagent. These traditional methods need harsh conditions and have poor functional group tolerance; therefore, the installation of the labeled carbon occurs at an early stage of the synthesis. An example of this is the synthesis of a potent and selective adenosine A_{2a} antagonist by Hesk et al. (**Fig. 1–11**).[84]

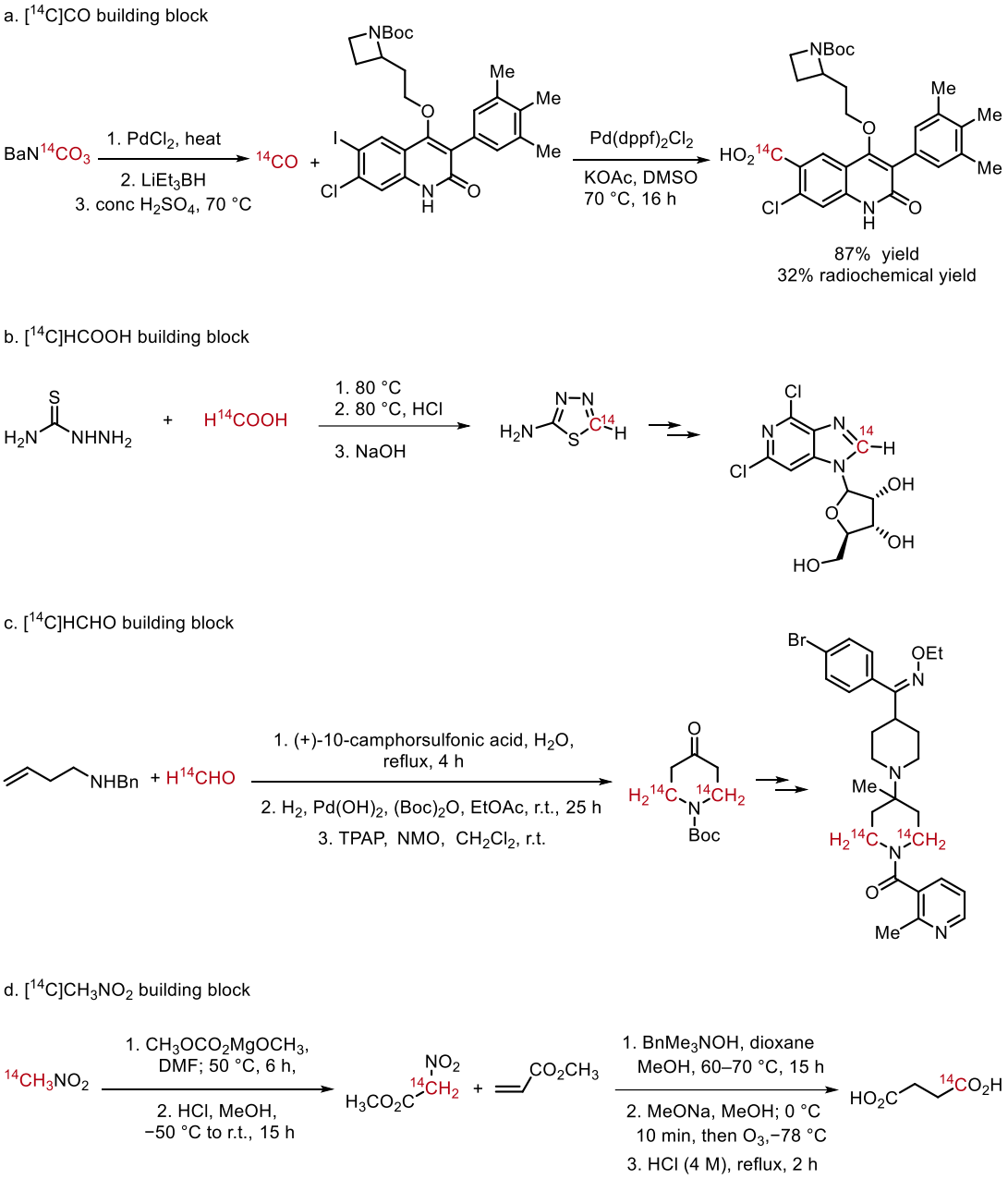


Fig. 1–10. $^{13/14}$C-Labeling of organic compounds with labeled building blocks: a. [^{14}C]CO building block, b. [^{14}C]HCOOH building block, c. [^{14}C]HCHO building block, d. [^{14}C]CH_3NO_2 building block.

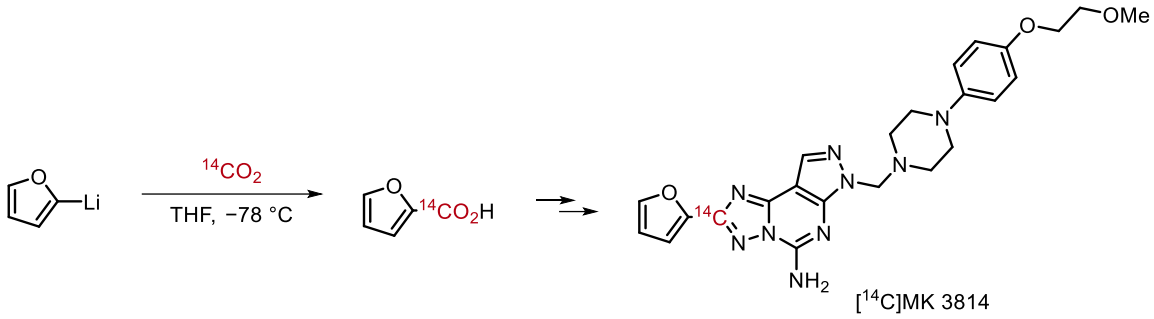


Fig. 1–11. Carboxylation of furan-2-yllithium route to [^{14}C]MK3814.

1.3.4.2 ^{11}C-Labeling of Organic Compounds

Carbon-11 has a half-life of 20.3 min. Carbon-11 is generally prepared via a cyclotron by proton bombardment of nitrogen gas. Carbon-11 labels are used as a radionuclide for positron emission tomography studies.[30] Incorporation of $^{13/14}$C into the organic molecule via functional group transformation can be done by using isotopically labeled building blocks.

Isotopically-labeled building blocks include the [^{11}C]CH_3X (X = I or OTf) building block. [^{11}C]CH_3I is by far the most frequently used ^{11}C-labeling agent, an example of which is the nucleophilic substitution of [^{11}C]CH_3X (X = I or OTf) to generate ^{11}C-methyl heteroatomic (N, O, or S) compounds. This simple and direct ^{11}C-methylating method has been well-applied in the synthesis of many ^{11}C-labeled tracers, including [^{11}C]PIB, [^{11}C]DASB, [^{11}C]flumazenil (**Fig. 1–12a**),[85] and the [^{11}C]CO building block. [^{11}C]CO is an established building block for the synthesis of ^{11}C-carbonyl labeled carboxylic acids, esters, amides, ketones, and aldehydes via transition-metal mediated carbonylation reactions. An example of this building block is the Pd-mediated transmetalation preceding ^{11}C-carbonylation with [^{11}C]CO. Representative examples include a retinoid compound, [^{11}C]Am80, and [^{11}C]aspirinobtained from the corresponding boronates (**Fig. 1–12b**).[86-87] Metal [^{11}C]cyanides building block (M = Na, K, Zn, Cu) are useful building blocks to introduce ^{11}C into an organic molecule. An example of that is the use of K[^{11}C]CN, as shown in **Fig. 1–12c**,[76] and the [^{11}C]CO_2 building block. [^{11}C]CO_2 is the feedstock virtually for all ^{11}C chemistry and can be converted readily to other synthons in high RCYs and high specific activity. An example of the use of this building block is when Riss and Pike et al. developed a novel

strategy to synthesize ^{11}C-labeled carboxylic acids and derivatives from boronic esters in the presence of a copper catalyst in high RCYs and high specific activity (**Fig. 1–12d**).[88]

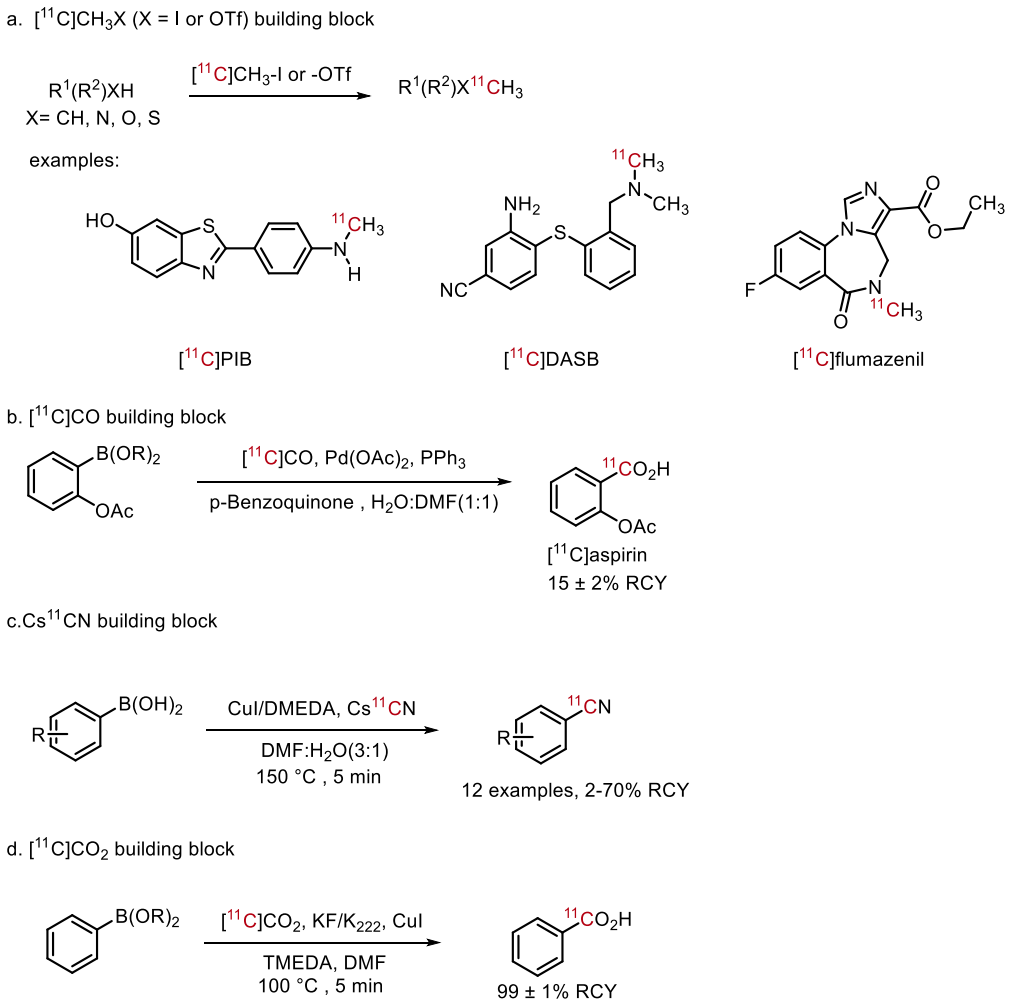


Fig. 1–12. ^{11}C-Labeling of organic compounds with labeled building blocks: a. [^{11}C]CH_3X (X = I or OTf) building block. b. [^{14}C]CO building block. c. Cs[^{11}C]CN building block. d. [^{11}C]CO_2 building block.

1.4 Decarboxylation and Carboxylation Reactions

1.4.1 Decarboxylation

A decarboxylation reaction is defined as a chemical reaction that involves removal of a carboxyl group. Decarboxylation is a fundamental step in biochemical processes and synthetic organic chemistry. Decarboxylation reactions can occur via several methods.

The first type is thermal decarboxyaltion, which happens upon heating certain carboxylic acids. Acid substrates that have strong anion-stabilizing groups adjacent to the reactive carbon center can decarboxlate readily at room tempreture (**Fig. 1–13a**); however, acid substrates lacking strong anion-stabilizing groups adjacent to the reactive carbon center are inert towards spontaneous decarboxylation without resorting to thermolysis conditions (**Fig. 1–13b**).[89-90]

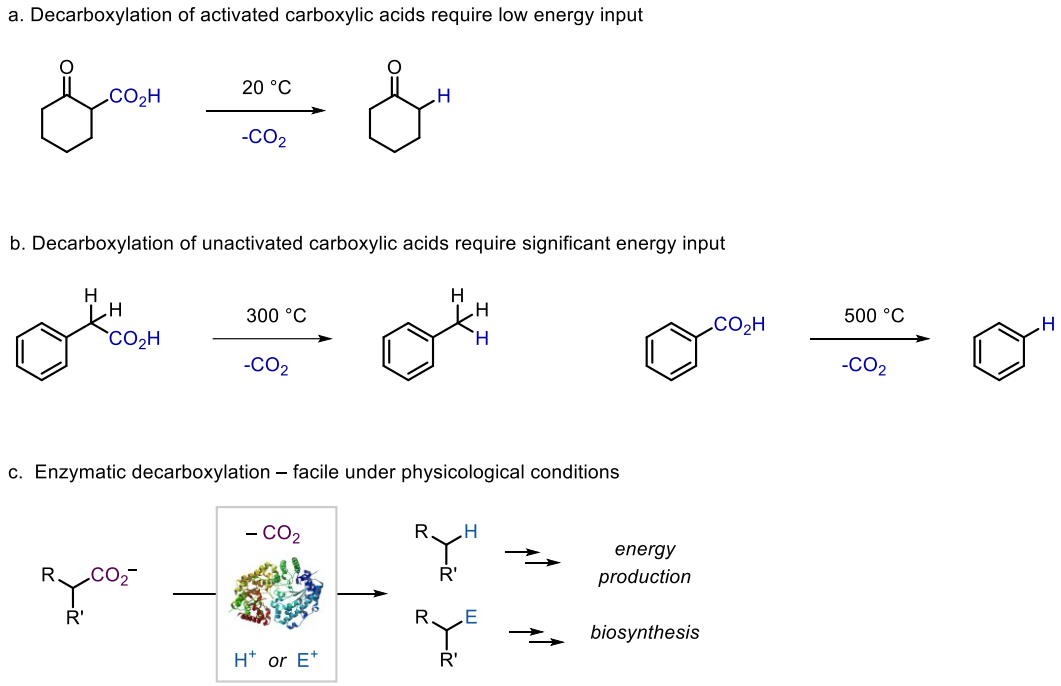


Fig. 1–13. a. Thermal decarboxylation of activated carboxylic acids, b. thermal decarboxylation of unactivated carboxylic acids, c. enzymatic decarboxylation.

The second type of decarboxylation is enzymatic decarboxylation, which utilizes decarboxylase enzymes. Fermentation, respiration, and the biosynthesis of many secondary metabolites involve the loss of CO_2 from organic acids.[91] Decarboxylase enzymes accelerate these reactions by stabilizing developing intermediates (typically carbanions) and promoting CO_2 diffusion from the active site, thereby enabling otherwise unfeasible decarboxylations to occur under physiological conditions (**Fig. 1–13c**).[92] The third type of decarboxylation is decarboxylation catalyzed by transition metals. Decarboxylation catalyzed by a transition metal complex to form a C–M bond is the critical step in decarboxylative cross-coupling

reactions. Several metals, such as Cu[93] (**Fig. 1–14a**), Pd[94] (**Fig. 1–14b**), and Ag[95] (**Fig. 1–14c** can catalyze decarboxylation). The fourth type is decarboxylation by single electron oxidation of carboxylate. This can occur via generation of NHPI ester (*N*-hydroxyphthalimide), followed by SET and decarboxylation (**Fig 1-14d, path I**), by SET to generate aryl carboxylic radical, followed by decarboxylation (**Fig 1-14d, path II**), or via generation of anhydride intermediate followed SET to get acyl radical (**Fig 1-14d, path III**).[96]

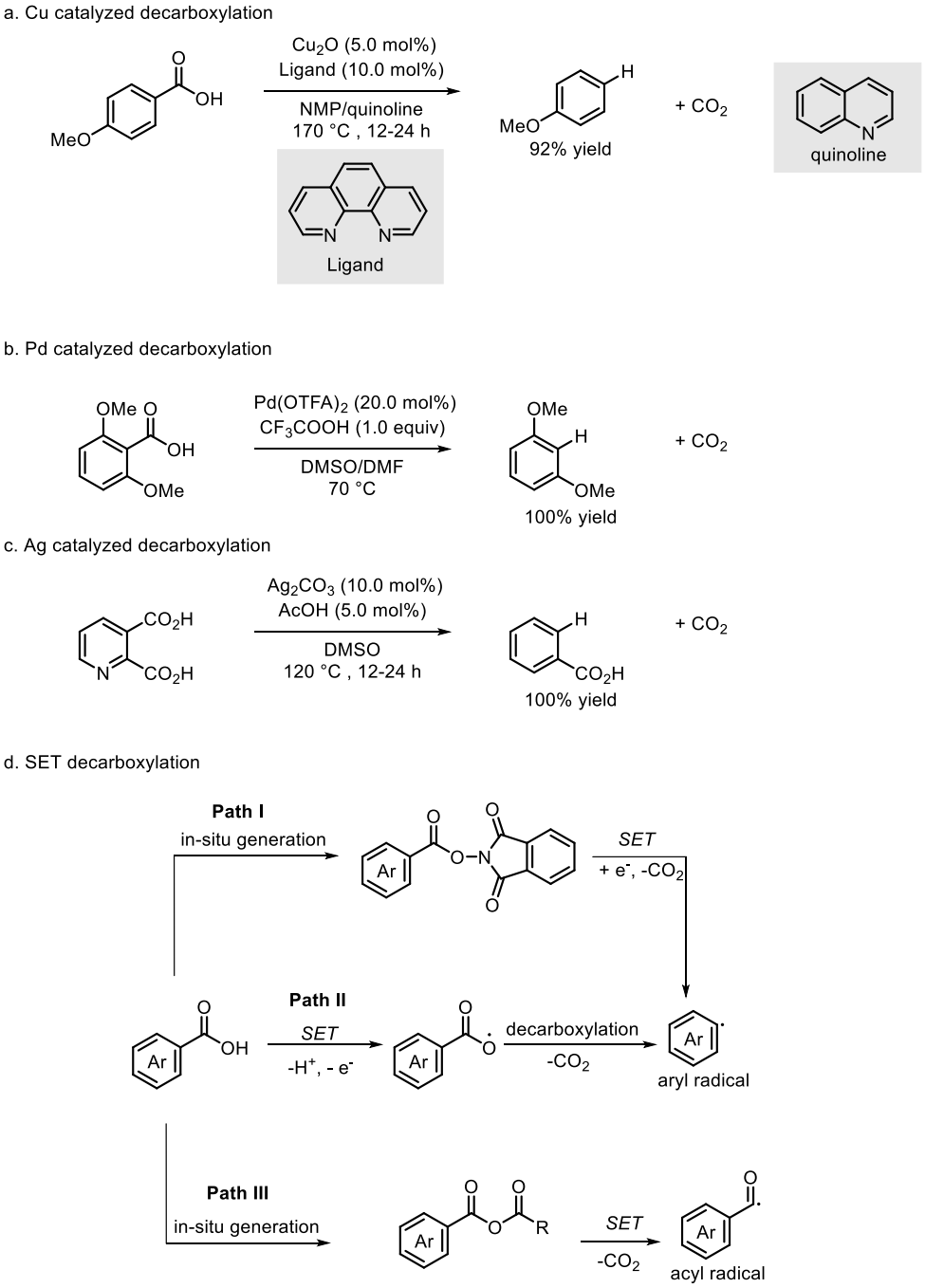


Fig. 1–14. a. Cu catalyzed decarboxylation, b. Pd catalyzed decarboxylation, c. Ag catalyzed decarboxylation, d. Decarboxylation by single electron oxidation of carboxylate.

1.4.2 Carboxylation

Carboxylation reactions, the microscopic reverse of decarboxylation, are equally valuable processes in biology and synthetic chemistry. Driven by the abundance and availability of CO_2, chemists were inspired to design different technologies for converting CO_2 into fine chemicals (**Fig. 1–15**).[97] One of the most attractive synthetic techniques for the utilization of CO_2 is to promote CO_2 conversion into carboxylic acids, which are building blocks in plastics, agrochemicals, and pharmaceuticals. Despite progress in this area, synthetic techniques to promote this process generally need to apply strongly nucleophilic organometallics and/or in-situ stoichiometric (electro)chemical substrate reduction.[97-98]

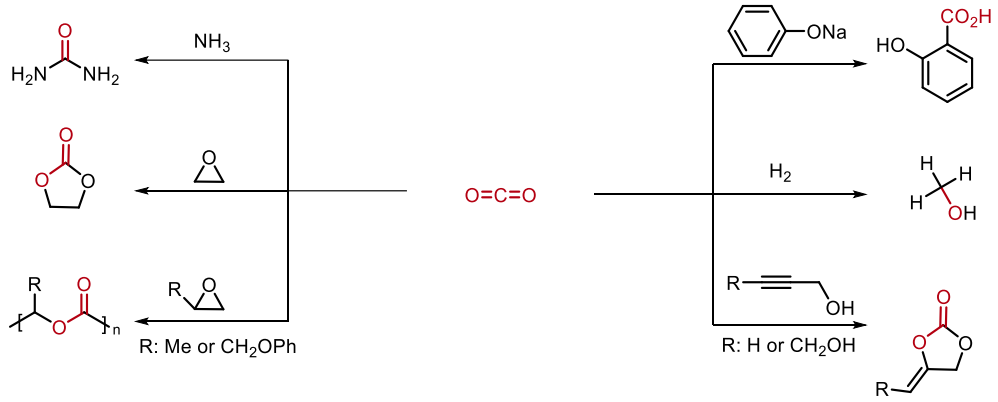


Fig. 1–15. Examples of industrial CO_2 fixation into chemicals.

1.5 Isotopic Labeling Via Exchange Reactions

1.5.1 Hydrogen Isotope Exchange (HIE) of Organic Compounds

The hydrogen isotope exchange (HIE) method for labeling organic molecules with deuterium or tritium is a more desirable method for the preparation of deuterium and tritium labeled compounds than labeling by functional group transformation because it leads to high isotopic yield, which can on occur on aromatic compounds (**Fig. 1–16a**),[99] olefins (**Fig. 1–16b**),[100] alkynes, aliphatic substrates, aliphatic alcohols and amines (**Fig. 1–16c**),[101] and aliphatic carbonyl compounds.[46]

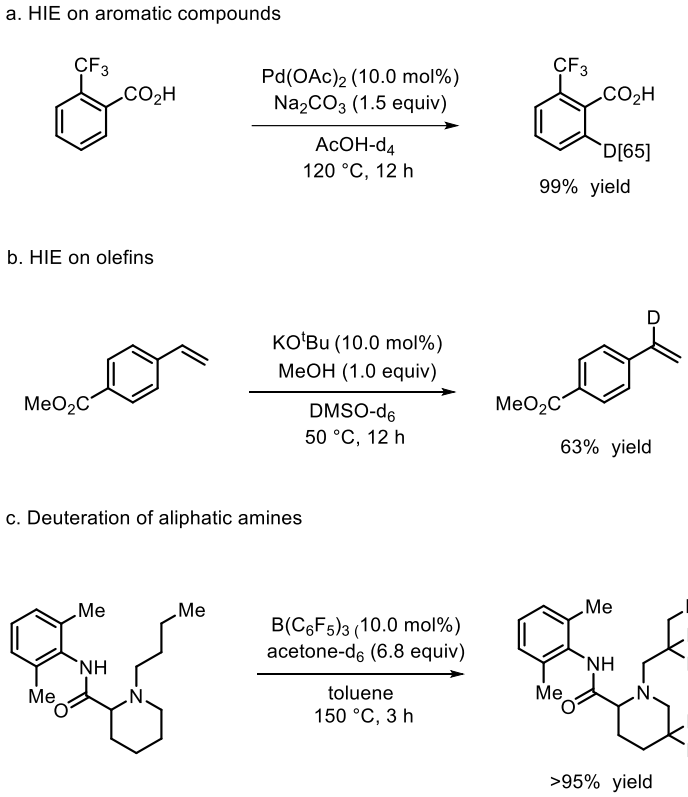


Fig. 1–16. Hydrogen isotope exchange (HIE) of organic compounds. a. HIE on aromatic compounds, b. HIE on olefins, c. deuteration of aliphatic amines.

Hydrogen isotope exchange (HIE) methods have attreacted great attention for labeling of pharmaceuticals compounds. Previously, H/D exchange carried out by a heterogeneous Pd/C–H_2–D_2O system was shown to be an efficient method for deuterium labeling of different types of alkyl-substituted aromatic compounds, including biologically active ibuprofen sodium salt (**Fig. 1–17**).[102]

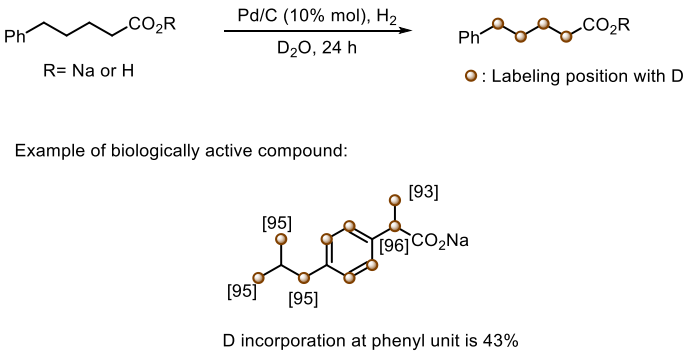


Fig. 1–17. Pd/C for the efficient deuteration of Ibuprofen sodium salt.

More recently, in 2016, Chirik and and co-workers reported the iron-mediated deuteration and tritiation of arenes and heteroarenes, revealing that H/D exchange is not limited to noble metals. They were able to apply their methodology for the labeling of drug molecules. For example, varenicline, papaverine, and loratadine worked under their methodology (**Fig. 1–18**).[103]

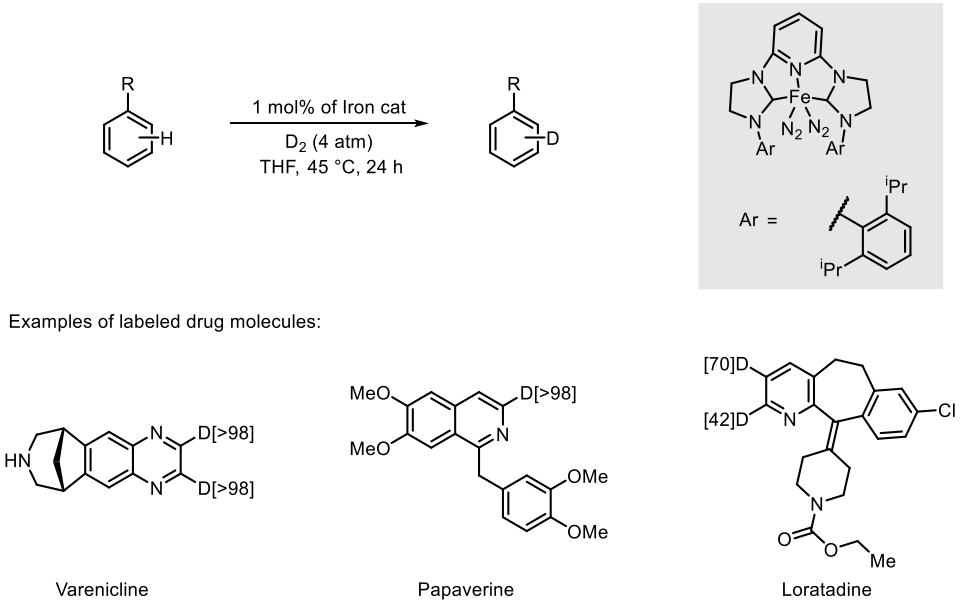


Fig. 1–18. Iron-mediated deuteration and tritiation of arenes and heteroarenes.

In 2017, MacMillan and co-workers successfully showed the use of photocatalysts in the α-deuteration and tritiation of complex pharmaceuticals. They were able to incorporate deuterium and tritium at α-amino sp^3 C–H bonds in a one pot strategy via a photoredox catalyst that mediates a hydrogen atom transfer (HAT) protocol, using isotopically labeled water (D_2O or T_2O) as the source of hydrogen isotope. Examples of that are the labeling of clomipramine, verapamil, escitalopram, and *cis*-diltiazem (**Fig. 1–19**).[104]

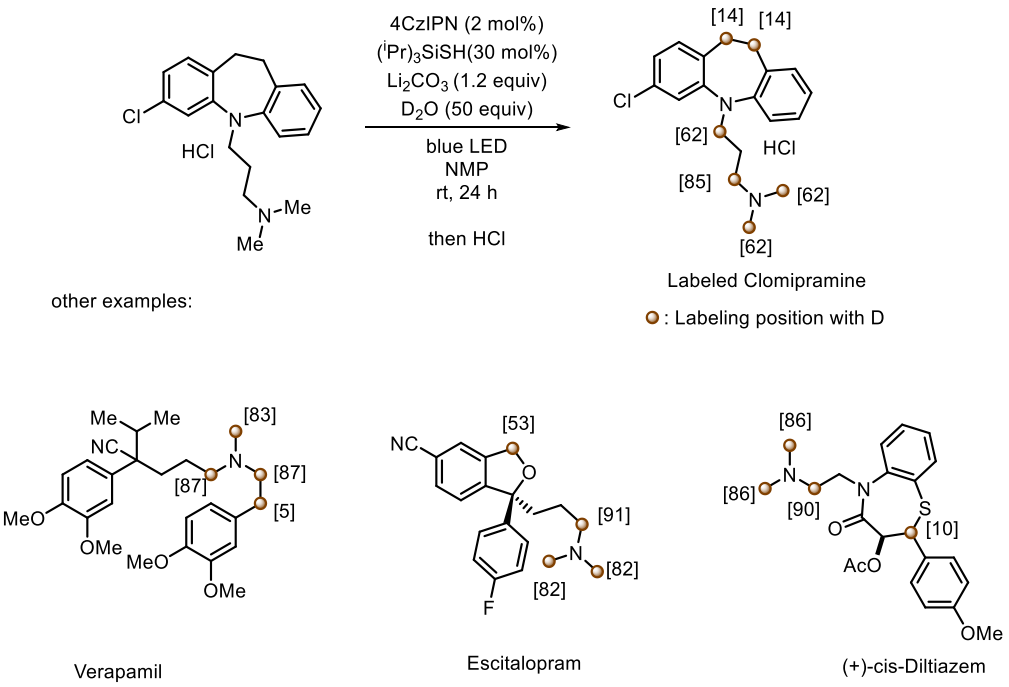


Fig. 1–19. The use of photocatalysts in the α-deuteration and tritiation of complex pharmaceuticals.

In 2019, Chirik and co-workers developed a methodology to enable labeling of a much broader selection of aromatic substrates. Using a specially-designed bulky, electron-donating diimine ligand and nickel hydride dimer, they were able to apply their methodology for the labeling of drug molecules, such as MK-6096 and varenicline (**Fig. 1–20**).[105]

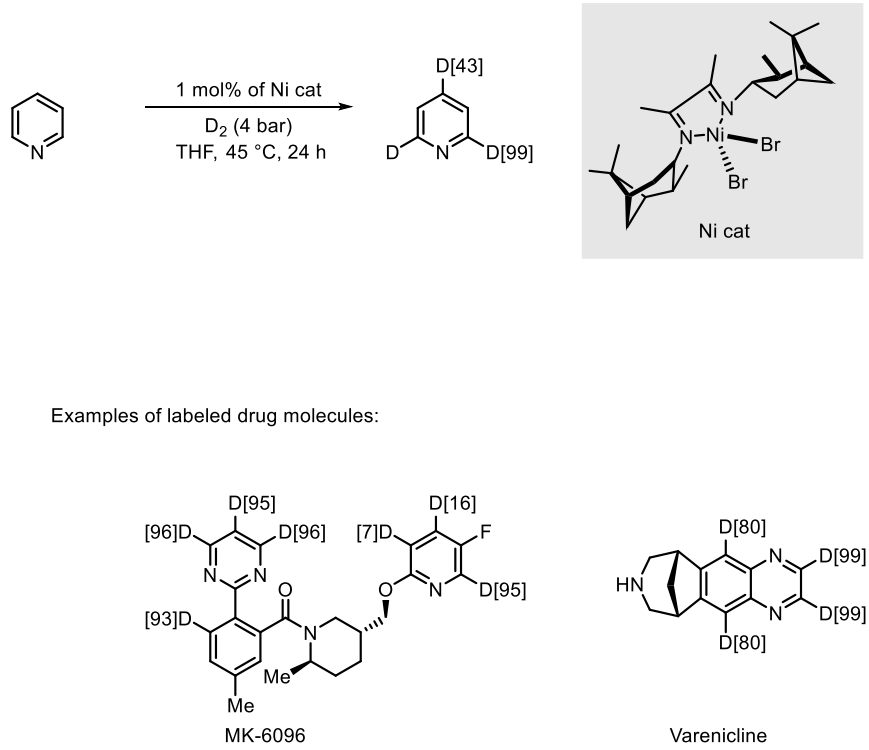


Fig. 1–20. The use of nickel hydride dimer in the α-deuteration and tritiation of complex pharmaceuticals.

More recently, in 2020 Pieters, Chaudret, Derdau, and co-workers demonstrated the utility of NHC-stabilized iridium nanoparticles for the more challenging HIE in anilines, using D_2 or T_2 as an isotopic source. They were able to apply their methodology for the labeling of diverse complex pharmaceuticals, like the drugs aminoglutethimide, sulfadimethoxine, sulfamoxole (**Fig. 1–21**).[106]

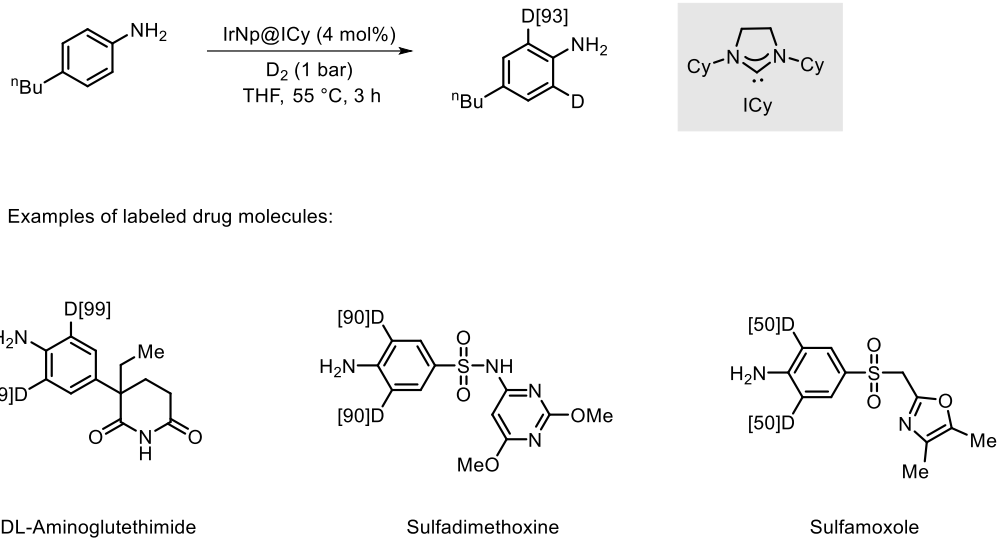


Fig. 1–21. The use NHC-stabilized iridium nanoparticles for HIE in anilines derivatives.

1.5.2 ^{12}C/*C Exchange of Organic Compounds [*C = ^{14}C, ^{13}C, or ^{11}C]

The incorporation of isotopically-labeled carbon via functional group transformation has some limitations. For example, some methods, like the carboxylation of organometallic compounds and coupling with organomagnesium or organolithium reagent, need harsh conditions and have poor functional group tolerance; therefore, the installation of the labeled carbon occurs at an early stage of the synthesis.

Another approach for the incorporation of isotopically-labeled carbon is via exchange reactions, which give an advantage to the late-stage incorporation of isotopically-labeled carbon. Reports of direct non-enzymatic reversible CO_2-exchange of carboxylic acids are restricted to specialized substrate/mediator pairs.[107] An example of that is when Darensbourg et al. showed that the dimeric complex $[(Ph_3P)_2CuO_2CCH_2CN]_2$ can undergo a reversible decarboxylation/carboxylation reaction readily in the temperature range 30–50 °C, as evidenced by its exchange with $[^{13}C]CO_2$ in DME (**Fig. 1–22a**).[108] The second method is the exchange of carboxylate groups in simple aliphatic acids with $[^{14}C]CO_2$, which has been documented but requires heating of neat substrates at 280–400 ºC (**Fig. 1–22b**).[109-110] The third method is the exchange of C(sp^2)-carboxylate groups. Destro et al. were inspired by HIE methods, which allow a radionuclide to be introduced on a previously synthesized drug in a single step. This methodology allows the direct exchange of C(sp^2)-carboxylate

groups catalyzed by transition metals. The reaction is limited to nitro- or sulfonyl-containing arenes or 2-heteroatom substituted electron-rich heterocycles at high temperature (≥150 ºC) (**Fig. 1–22c**).[111] The fourth method is by chemical activation-decarboxylation-metalation-carboxylation sequences mediated by transition metals.[112-114] An example of this approach is when Baran et al. reported the nickel-mediated decarboxylative carboxylation of alkyl carboxylic acids via redox-active ester formation (**Fig. 1–22d**).[113] The fifth method of $^{12}C/^{*}C$ involves the use of labeled carbon monoxide in place of CO_2.[114-115] As an example of this method, Gauthier et al. have reported the use of COgen as a source for labeled carbon monoxide to promote palladium-catalyzed [^{13}C]CO and [^{14}C]CO exchange with activated aliphatic and benzoic carbonyls (**Fig. 1–22e**).[116]

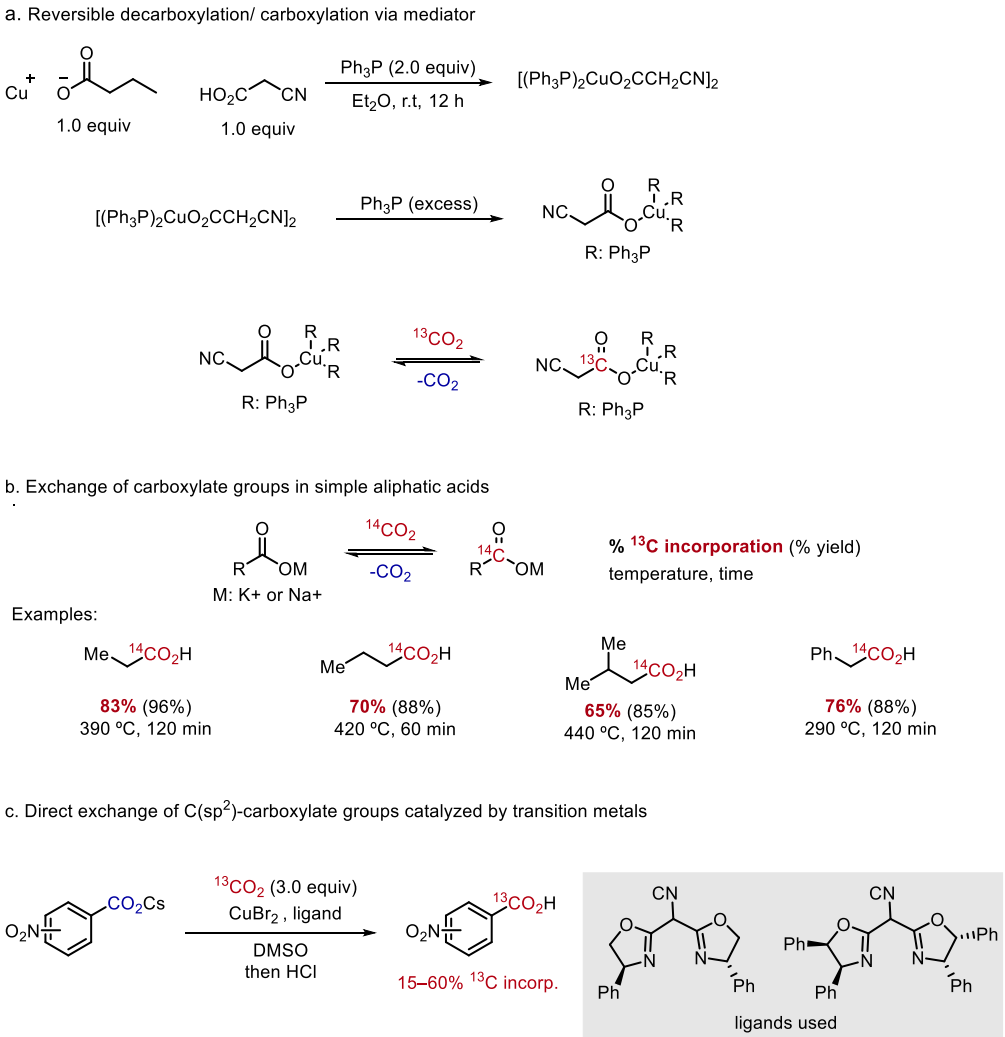


Fig. 1–22. Methods for introducing labeled carbon via exchange reactions.

1.6 Thesis Overview

This thesis describes the discovery, optimization, and application of new methods for the isotopic labelling of stabilized carboxylic acids and α-amino acids with *CO_2 (* = 14, 13, 11). Mechanistic studies of the processes were conducted, with a particular focus on elucidating the nature of the decarboxylation steps. Our group developed methods for catalytic decarboxylative cross-coupling reactions. The main inquiry that always arises in these methods is about the nature of the decarboxylation event, whether it is reversible or not. So, we were motivated to answer this question and investigate the nature of the decarboxylation event for stabilized carboxylic acids and their derivatives, which would enable the incorporation of labeled *CO_2 (* = 14, 13, 11) if the decarboxylation event is reversible.

Chapter 2 discusses the synthesis of isotopically-labeled carboxylic acids via direct reversible decarboxylation of stable organic acids in a polar aprotic solvent. This process involves uncatalyzed reversible decarboxylation/carboxylation in dimethylformamide solution to trap [^{13}C]CO_2 electrophile to generate isotopically labeled carboxylic acids. It displays a wide tolerance to isotopic labeling processes due to the use of mild conditions and simple protocol.

Chapter 3 discusses the synthesis of isotopically labeled racemic α-amino acids, using achiral aldehyde catalysis in conjunction with isotopically-labeled CO_2. Proteinogenic α-amino acids and many non-natural variants containing diverse functional groups undergo labeling. The reaction likely proceeds via the trapping of *CO_2 by imine-carboxylate intermediates to generate iminomalonates that are prone to monodecarboxylation. The pre-generation of the imine carboxylate intermediate allows for the rapid and late-stage ^{11}C-radiolabeling of a-amino acids in the presence of [^{11}C]CO_2.

Chapter 4 discusses the synthesis of enantioenriched isotopically labeled α-amino acids using a chiral aldehyde auxiliary in conjunction with isotopically labeled CO_2. Most proteinogenic α-amino acids and many non-natural variants containing diverse functional groups undergo labeling with good enantiomeric ratio (~90:10) and, with the knowledge learned from previous mechanistic studies (Chapter 3), proved to be invaluable in the development of the process.

Chapter 5 provides a brief overview of the research objectives accomplished in this thesis as well as providing some possibilities for future work which was inspired by the findings in this thesis.

CHAPTER 2

Synthesis of Isotopically-Labeled Carboxylic Acids via Direct Reversible Decarboxylation of Stable Organic Acids in a Polar Aprotic Solvent

2.1 Introduction

Extrusion of CO_2 is an important step in organic synthesis since many organic synthetic methods depend on CO_2 extrusion to generate reactive intermediates for bond-forming events. Decaboxylation is an important step in biochemical processes and organic chemistry because many biochemical processes, such as fermentation, respiration, and the biosynthesis of many secondary metabolites, involve the loss of CO_2 from organic acids.[91] Carboxylic acids substrates having strong anion-stabilizing groups adjacent to the reactive carbon center, such as β-oxoacids, diphenylacetic acids, and polyfluorobenzoic acids, can readily decarboxylate even in the absence of metals at moderate temperatures **(Fig. 2–1a)**.[117] Acid substrates lacking strong anion-stabilizing groups adjacent to the reactive carbon center have been construed to be inert towards spontaneous decarboxylation. Decarboxylation of these acid substrates requires subjecting them to thermolysis conditions.[89-90] Examples of this class include decarboxylation of phenylacetic acid[89] and benzoic acid[90] at very high temperatures, as shown in **Fig. 2–1b**. As a result, synthetic reactions driven by decarboxylation of these acid substrates are carried out using high reaction temperatures[117] or added oxidizing agents.[118-119] An oxidant can promote decarboxylation by single electron oxidation of carboxylate (SET), followed by decarboxylation (**Fig 2-1c**), or prior stoichiometric chemical modification of the carboxylate unit.[120-122]

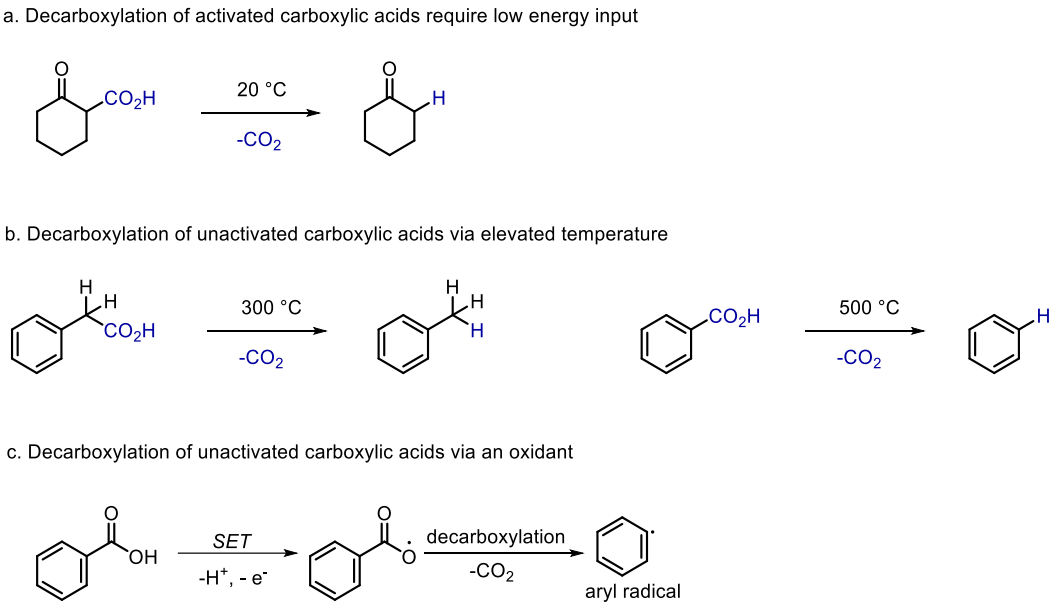


Fig. 2–1. Decarboxylation of activated and unactivated carboxylic acids.

Carboxylation is an important step in organic synthesis because of the availability and abundance of CO_2. The most common application of the use of CO_2 in carboxylation reactions is the conversion of CO_2 into carboxylic acids, which are building blocks in plastics, agrochemicals, and pharmaceuticals.[97] Despite the development in this area, synthetic techniques to promote this process need to apply strongly nucleophilic organometallics and/or in-situ stoichiometric (electro) chemical substrate reduction.[97-98]

The potential for the reversibility of decarboxylation/carboxylation mechanisms often is ignored in reports of synthetic methodologies that rely on these elementary steps. Reports of non-enzymatic direct reversible CO_2-exchange of carboxylic acids are restricted to specialized substrate/mediator pairs **(Fig. 2–2a)**.[107-108] The exchange of carboxylate groups in simple aliphatic acids with CO_2 has been documented, but it requires the heating of neat substrates at 280–440 ºC **(Fig. 2–2b)**.[109-110]

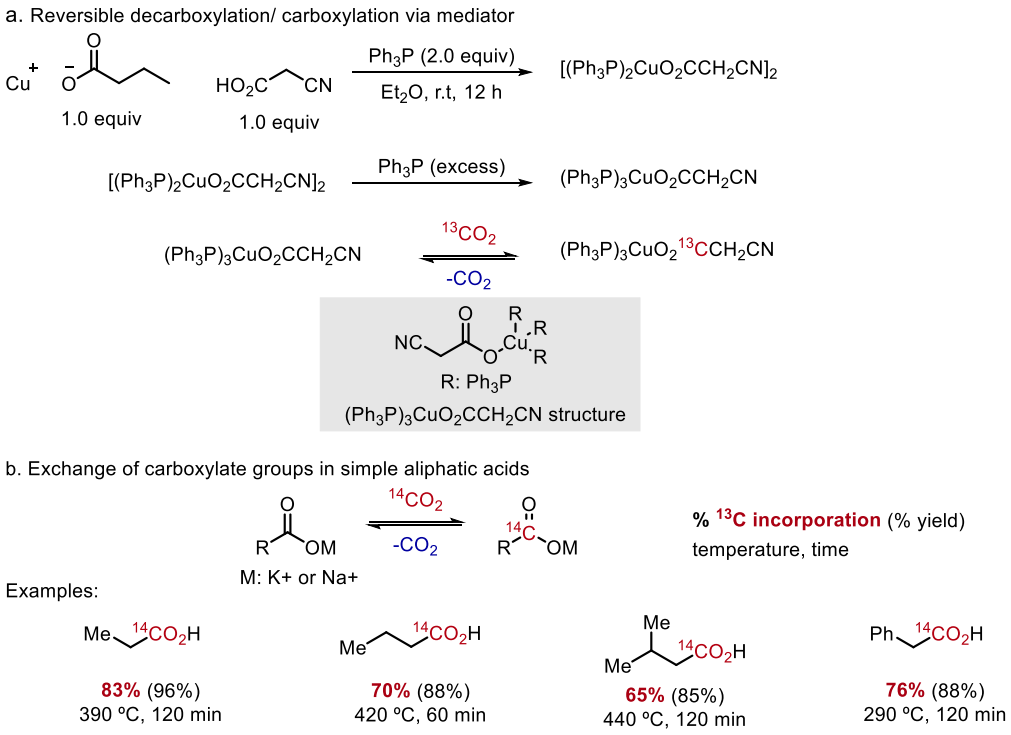


Fig. 2–2. Reports for reversibility of decarboxylation/carboxylation mechanisms.

Isotopic labeling of molecules with isotopes of carbon is important because labeling molecules with isotopes of carbon is sought widely in many disciplines (medicinal chemistry, agroscience, PET studies).[24-30] The use of carbon labels is often the ideal labeling approach because the molecule's scaffolds are not altered significantly (like they are with ^{18}F labels) and are not prone to wash out or metabolic shifting (like with ^{2}H or ^{3}H labels).[13]

Isotopic labeling of organic molecules by incorporation of a labeled carbon C* (*C = ^{14}C, ^{13}C, or ^{11}C) via exchange reactions has been discussed in chapter 1 (**Fig 1-22**). Recently, in concert with our work, Destro et al. developed isotopic exchange of carboxylate groups in cesium arylacetic acid salts with $*CO_2$ (*C =^{14}C, ^{13}C, or ^{11}C) in DMSO at 80–190 ºC (**Fig. 2–3**).[123]

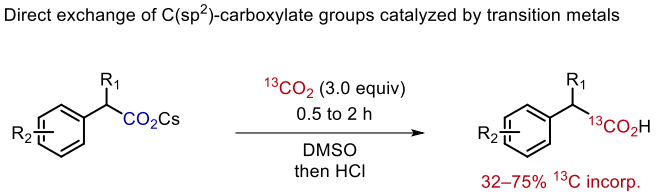


Fig. 2–3. Direct exchange of C(sp^2)-carboxylate groups catalyzed by transition metals.

Our group developed methods for catalytic decarboxylative cross-coupling reactions. The main inquiry that always arises in these methods is about the nature of the decarboxylation event.[124-125] So, we were motivated to answer this question and investigate the nature of the decarboxylation event. Section 2.2 describes the development of the first direct reversible decarboxylation of stable organic acids in a polar aprotic solvent to access isotopically-labeled carboxylic acids.

2.2 Development of Carboxylate Exchange for Stable Organic Acids in Conjuction with Isotopically-Labeled CO_2

2.2.1 Discovery and Development of the Methodology

We were inspired to know whether the nature of a decarboxylation event was reversible or not. In a decarboxylative amination project,[126] Patrick Moon, a graduate student in our group, ran a mechanistic study to investigate this, and he noticed that the loss of CO_2 from activated aryl acetate is reversible. Thus, we decided to examine the generality of this concept on other stabilized carboxylic acids.

Starting from the reaction conditions that he observed in the decaboxlyation amination project,[126] Moon showed that the potassium salt of arylacetic acid (**2-1**) exemplifies the reversible decarboxylation/carboxylation behavior in a polar aprotic solvent. He carried out the reaction in a glovebox filled with N_2, then he added DMF (1 mL) to a dried 4-dram vial charged with a stir bar and the potassium salt of arylacetic acid (0.1 mmol, **2-1**). The vial was sealed with a PTFE-lined cap and removed from the glovebox. The reaction headspace was evacuated on a Schlenk line (~300 mtorr) using a 25-gauge needle. Then, the vial headspace was refilled carefully with 15 psi [^{13}C]CO_2 through the PTFE-lined cap with a 25-gauge needle until the internal pressure reached ~1 atm (requires 20–60 seconds, depending on the pressure of the [^{13}C]CO_2 tank). This provides ~8 equivalents (~0.8 mmol) of [^{13}C]CO_2, which would result in an equilibrium exchange incorporation of ~85%; the equivalence of [^{13}C]CO_2 was approximated according to the headspace volume of the vial, using the ideal gas law. The equivalencies are calculated as follows: to get equivalencies we need to know the # of mmols [^{13}C]CO_2, so we solve for n:

$$pV = nRT$$

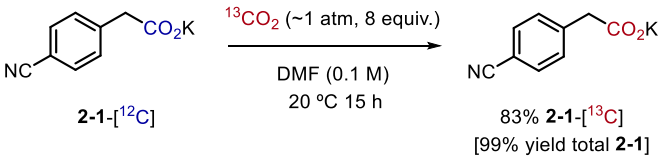
$$1\text{ atm } 0.0195\text{ L} = n\ 0.0821\text{ L atm mol}^{-1}\text{ K}^{-1}\ 298\text{ K}$$

n = 0.8 mmol (~8 equivalents)

In a reaction where approximately eight equivalents of [^{13}C]CO_2 were supplied (19.5 mL of CO_2 at ~1 atm, dissolved [CO_2] = 0.20 M), equilibrium between ^{12}C and ^{13}C was achieved in 15 h (**Fig. 2–4a**). Quantitative recovery of carboxylate (**2-1**) with 83% ^{13}C-enrichment was possible by acid/base extractive workup.

Duanyang Kong, a postdoc in our group, investigated the effect of other counter-cations and solvents on the reactivity of carboxylate exchange (**Fig. 2–4b**). The carboxylic acid of (**2-1**) manifested no evident [^{13}C]CO_2 exchange or protodecarboxylation in DMF at 70 ºC. Li^+ , and Na^+ salts of (**2-1**) reacted more slowly, while the Cs^+ salt reacted more quickly. Divalent metal salts of **1** (Zn^{2+} or Cu^{2+}) were inert to exchange conditions. Also, he studied the carboxylate exchange reactivity in different solvents, and he found that the use of polar aprotic solvents (DMF, DMA, DMSO; dielectric constant $\varepsilon > 30$) is essential for the transformation: Reactions conducted in THF, DCE, or water resulted in the recovery of unlabeled **2-1** at 20 ºC. The addition of 18-crown-6 (18-C-6) led to an approximate two-fold rate enhancement of carboxylate exchange.[127]

a. Reversible decarboxylation/carboxylation of cyanophenylacetate



2-1-[^{12}C]

83% **2-1**-[^{13}C]
[99% yield total **2-1**]

b. Impact of salt and reaction conditions

cation	^{13}C incorp. (time)
K^+	85% (1 h)
H^+	<5% (8 h)
Li^+	72% (8 h)
Na^+	77% (8 h)
Cs^+	63% (5 h, rt)
0.5 Zn^{2+}/Cu^{2+}	<5% (8 h)

conditions	^{13}C incorp. (time)
THF	0% (5 h)
DCE	0% (5 h)
H_2O	0% (5 h)
DMSO	47% (5 h)
DMA	45% (5 h)
DMF/18-C-6	67% (5 h)

Fig. 2–4. Overview of decarboxylative processes and carboxylate exchange: a. reversible decarboxylation/carboxylation of cyanophenylacetate (2-1), b. impact of salt and reaction conditions.

2.2.2 Scope of the Methodology

The direct reversible decarboxylation of stable organic acids in a polar aprotic solvent process enables access to isotopically labeled carboxylic acids. An overview of the scope examples carried out by Duanyang is provided in **Fig. 2–5**. He was able to show that (hetero)arylacetic acid salts with anion-stabilizing groups can undergo carboxylate exchange at moderate temperatures (**2-1**, **2-2**, **2-3**, **2-4**, **2-5**, **2-6**, **2-7**), whereas arylacetates with a strongly electron-donating OMe group required higher temperatures (**2-8**, **2-9** at 100 to 115 ºC) and benefitted from the addition of 18-C-6. Alkyl and aryl substitution adjacent to the carboxylate was tolerated, including examples of disubstituted arylacetic acid salts (**2-10**) and trisubstituted, non-enolizable arylacetic acid salts (**2-11**, **2-12**). The simplicity of the process enabled broad functional group compatibility, including tolerance to ketones (**2-4**), aldehydes (**2-5**), amides (**2-6**), esters (**2-7**), and potentially reactive heterocycles (pyridines **2-14**, **2-15**, pyrimidine **2-16,** isoxazole **2-16**). He showed also that other classes of potassium carboxylates can undergo reversible decarboxylation, including malonate half-esters (**2-17, 2-18**), and cyanoacetates (**2-19**).

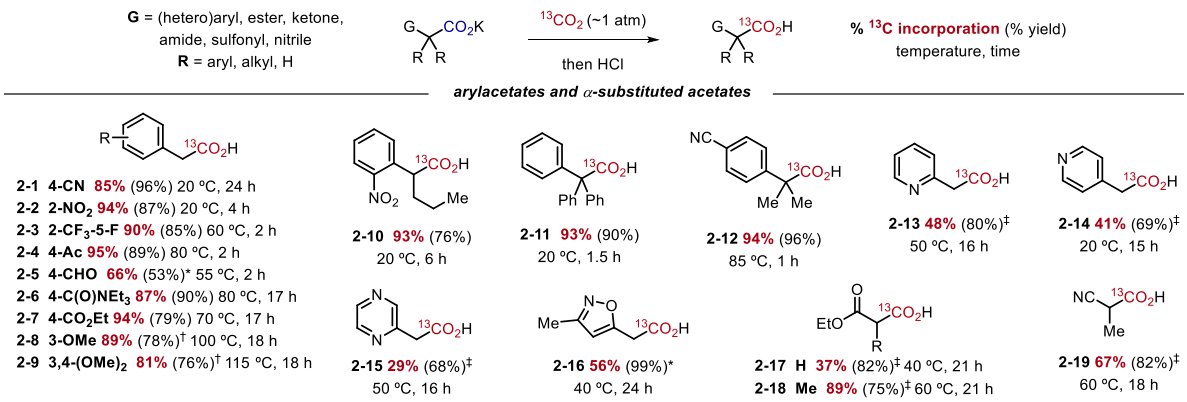


Fig. 2–5. Carboxylate exchange scope**.** Unless noted yields are of isolated material. *Calibrated ^{1}H NMR spectroscopy yield using 1,3,5-trimethoxybenzene as an internal standard. †1.0 Equivalent 18-C-6 added. ‡%^{13}C Incorporation and yield determined by analysis of the corresponding methyl or benzyl ester.

I was able to expand the scope of the reaction, as shown in **Fig. 2–6**, to include (hetero)arylacetic acid salts with anion-stabilizing groups, which can undergo exchange exchange at moderate temperatures (**2-20**, **2-21**, **2-22, 2-26**) as well as arylacetates with strongly electron-donating OMe or NMe_2 groups that required higher

temperatures (**2-29**, **2-30** at 130 to 140 ºC) and benefitted from the addition of 18-C-6. The simplicity of the process enabled broad functional group compatibility, including tolerance to boronic esters (**2-23**), aryl halides (I, Br, Cl, F; **2-21**, **2-24**, **2-25**, **2-3**), sulfonyls (**2-27**), and potentially reactive heterocycles (chromenone **2-32,** NH-indole **2-33**, and thiophene **2-34**). I also showed that the other classes of potassium carboxylates can undergo reversible decarboxylation, including β-carboxysulfonyls (**2-35**, **2-36**) and malonate half-esters (**3-37**, **2-38**). Alkene and terminal alkyne functional groups did not interfere with the process (**2-35**, **2-36**). Potassium malonates underwent CO_2 exchange at higher temperature (135 ºC) to give a mixture of mono- and doubly-labeled product along with ^{13}C-enriched monoacid (**2-39**, **2-40**).

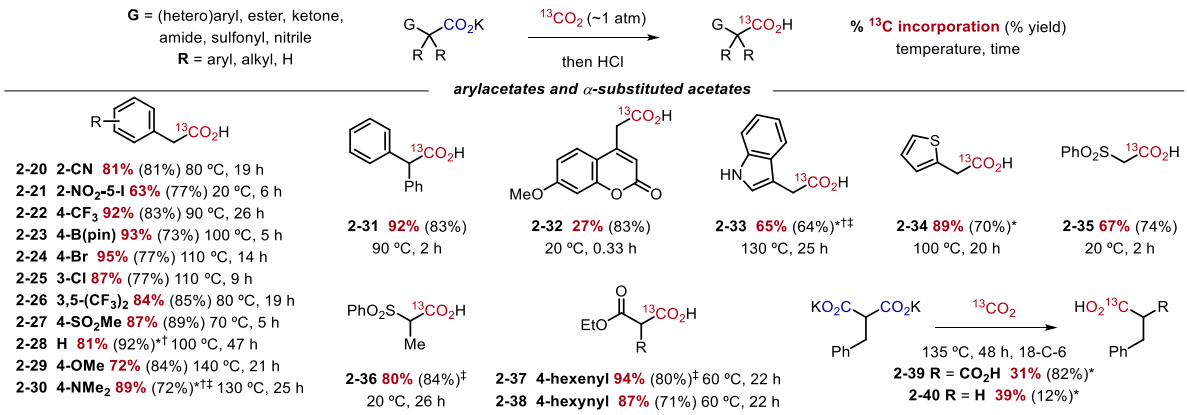


Fig. 2–6. Expansion of carboxylate exchange scope. Unless noted, yields are of isolated material. *Calibrated ^1H NMR spectroscopy yield using 1,3,5-trimethoxybenzene as an internal standard. †1.0 Equivalent 18-C-6 added. ‡%^{13}C Incorporation and yield determined by analysis of the corresponding methyl or benzyl ester.

2.2.3 Carboxylate Exchange/Derivatization to Access Alkyl Carboxylic Acids

Simple alkyl carboxylates did not undergo CO_2 exchange (**Fig. 2–7a**) under our standard reaction conditions because they lack strong anion-stabilizing groups adjacent to the reactive carbon center. However, isotopically-labeled products of this class can be obtained readily by carboxylate exchange/desulfonylation reations of β-sulfonyl acids (**Fig. 2–7b**) or exchange/decarboxylation sequences of malonic acids in three steps (**Fig. 2–7c**).

We also were able to show that the potassium salt of diphenylmethylidene glycine can undergo reversible exchange at room temperature to access [C_1-

^{13}C]diphenylmethylidene glycine (93% incorporation, 76% yield) (**Fig. 2–7d**). The facile generation of [C$_1$-^{13}C]diphenylmethylidene glycine serves as a starting point for the synthesis of other labeled amino acids.

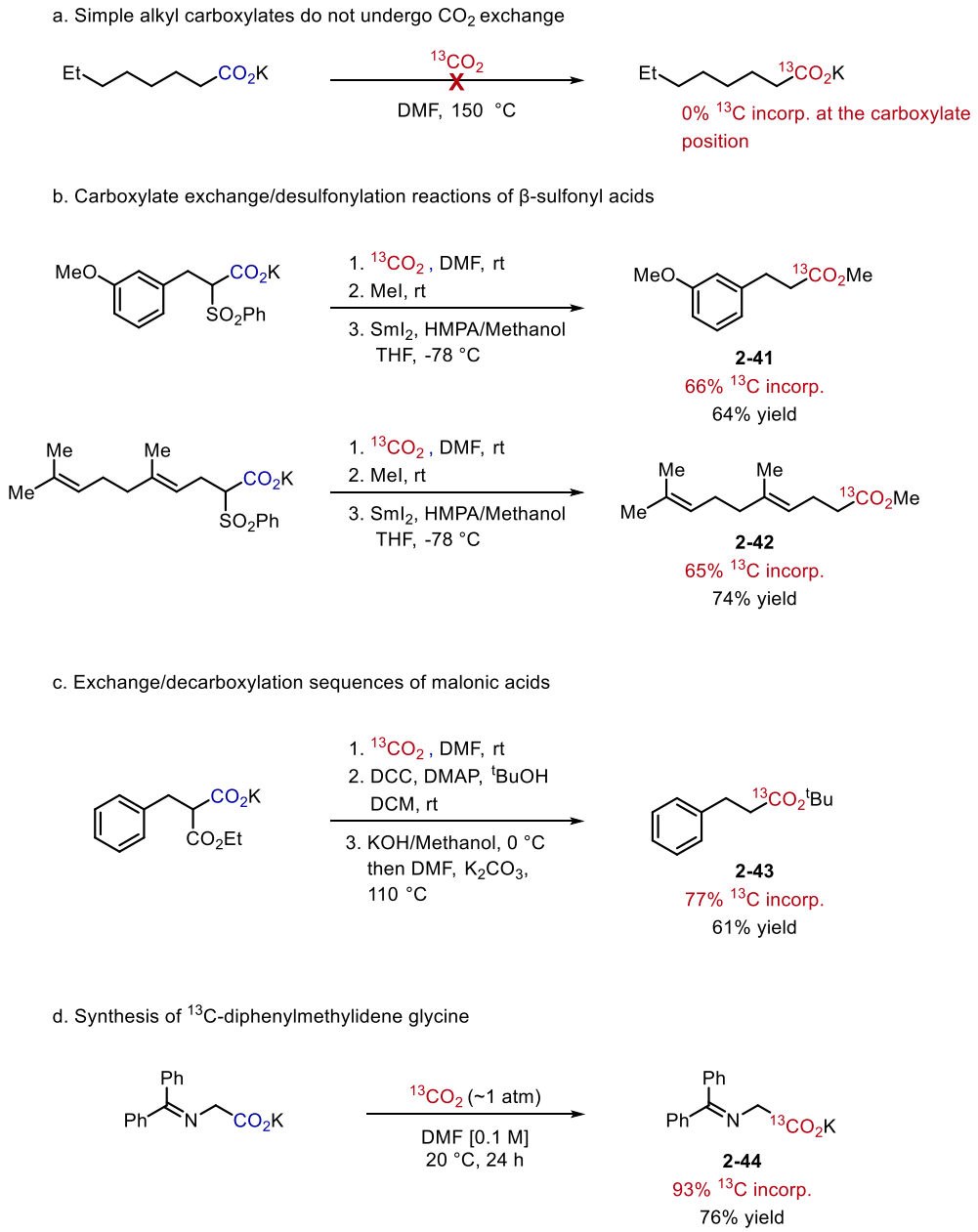


Fig. 2–7. Derivatizations and synthetic intermediates.

2.2.3 Mechanistic Studies

The reversible CO_2 exchange process likely involves the formation of a carbon nucleophile either from direct decarboxylation or, potentially, in the case of enolizable substrates, through an enolate intermediate. Duanyang showed evidence for the potential of direct decarboxylation as substrates with no alpha hydrogen (**2-11**, **2-12**) worked well under the standard conditions (**Fig. 2–7a**). He also showed evidence for a dienolate nucleophile, such as the potassium salt of arylacetic acid (**2-1**) at 70 ºC under N_2, which underwent slow net carboxylate/proton metathesis to generate a half equivalent of the protodecarboxylated product (**2-44**) and a half equivalent of the CO_2-trapped malonate (**2-45**) **(Fig. 2–7b**). Product (**2-44**) likely arises from deprotonation of a second equivalent of aryl acetate to generate a dienolate nucleophile. The dienolate intermediate can react with the CO_2 released by the initial decarboxylation event. Also, he showed that carbonic anhydride intermediates likely are generated under the reaction conditions on the basis of the observed increase in α-carboxyl H/D exchange rates with 1-H_2 and 1-D_2 under CO_2 (**Fig. 2–7c**).

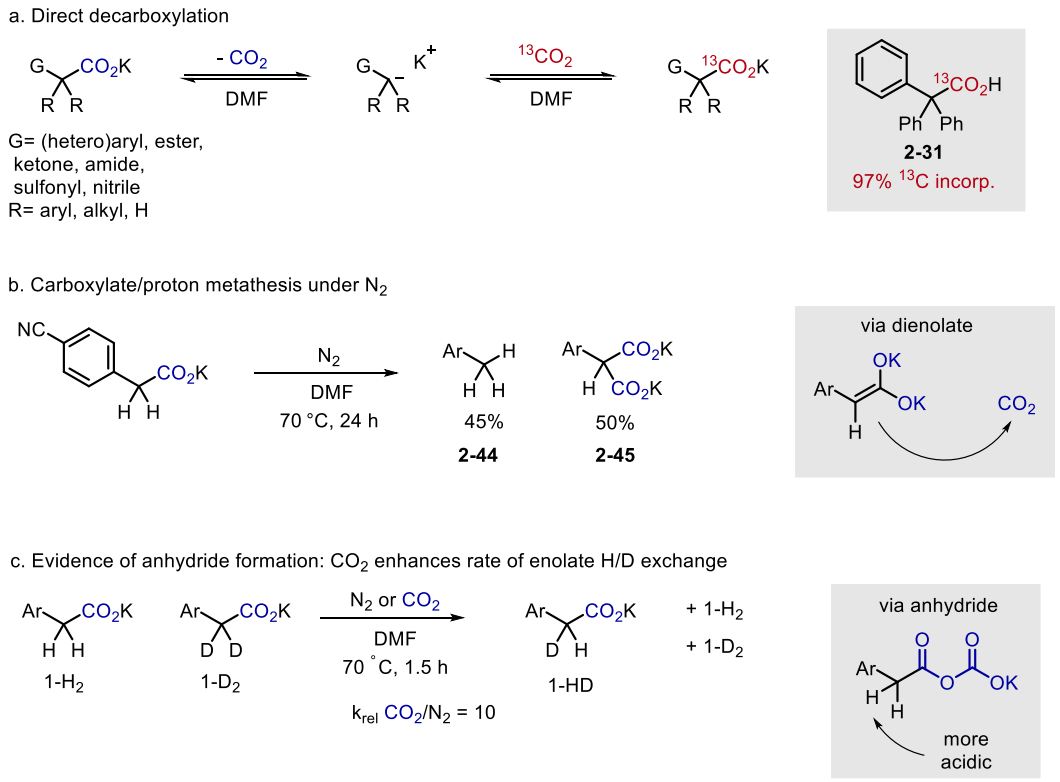


Fig 2–8. Mechanistic control experiments.

2-3 Summary and Conclusions

In summary, we showed that stable carboxylates can undergo reversible decarboxylation/carboxylation in a polar aprotic solvent. We have utilized this finding to develop a new strategy for the isotopic labeling of activated carboxylic acids with isotopically-labeled [^{13}C]CO_2, based on a reversible decarboxylation/carboxylation event of stable carboxylates. Our developed methodology has several merits, including the broad scope of reactivity, broad functional-group tolerance, simple protocol, cost effectiveness, and is a transition metal-free methodology.

We also showed that diphenylmethylidene glycine can undergo reversiable exchange at room temperature, which serves as a starting point for the synthesis of other labeled amino, acids as will be discussed in Chapter 3.

2.4 Procedures and Characterization

General Considerations

Unless noted, all reactions were conducted under inert atmosphere employing standard Schlenk techniques or by the use of a N_2-filled glovebox. All glassware was oven-dried prior to use. Flash chromatography was performed, as described by Still and co-workers[128] (SiliaFlash P60, 40-63μm, 60A silica gel, Silicycle) or by automated flash chromatography (SiliCycle silica cartridges, Biotage). Analytical thin-layer chromatography was performed, using glass plates pre-coated with silica (SiliaPlate G TLC - Glass-Backed, 250 μm, Silicycle). HPLC analysis was accomplished on an Agilent 1290 system with Daicel CHIRALPAK IA, IB, IC or IG columns (4.6 x 150 mm, 5 μm particle size). NMR spectra (^{1}H, ^{13}C, ^{19}F) were obtained on an Agilent VNMRS 700 MHz, Varian VNMRS 600 MHz, Varian VNMRS 500 MHz, or Varian 400 MHz spectrometer. HRMS analyses of carbon-13 labeled compounds were performed on an Agilent Technologies 6220 oaTOF instrument (ESI, APPI and APCI) or a Kratos Analytical MS-50G instrument (EI) in positive or negative ionization mode. DMF and [^{13}C]CO_2 (99.0 atom % ^{13}C) were purchased from Sigma-Aldrich. The corresponding aryl acetic acids were obtained from commercial vendors. Potassium aryl acetate salts (**2-30**, **2-13**, and **2-14**) were synthesized from the corresponding aryl acetic esters, as described by Liu and co-workers.[130] Malonate half ester salts (**2-37** and

2-38) were synthesized according to the literature procedure from the corresponding malonate esters.[130,131] *N*-(diphenylmethylene)glycinate potassium **2-44** was synthesized from ethyl *N*-diphenylmethylene glycine, as described by Alezra and co-workers.[132] Other aryl acetate salts were synthesized from the corresponding aryl acetic acids, as described by Liu and co-workers.[131]

General Procedures and Characterization for Section 2.2

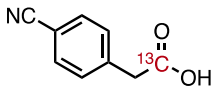
General Procedure A: In a glovebox filled with N_2, potassium carboxylate salt (0.1 mmol, 1.0 equiv), 1,3,5-trimethoxybenzene (an internal standard), and anhydrous DMF or DMSO (1.0 ml) were added sequentially to a dried 4-dram vial charged with a stir bar. The potassium carboxylates are moderately hygroscopic and best stored under inert atmosphere, however, a glovebox is not required. The vial was sealed with a PTFE-lined cap and removed from the glovebox. The reaction headspace was evacuated (~ 300 mtorr), via a 25-gauge needle. Next, the headspace was refilled carefully with ~1 atm [^{13}C]CO_2 through the PTFE cap, using a 25-gauge needle, and stirred at the corresponding temperature. Reactions at elevated temperatures were heated in an aluminum block. ^{1}H NMR or LC-MS analysis of small aliquots (~5 μL) was used to follow reactions. Optimal temperatures for carboxylate exchange without re-charging of [^{13}C]CO_2 could be found by adjusting the temperature 10–20 °C every hour until ^{13}C incorporation was observed. Upon the desired incorporation of the ^{13}C-isotope, the mixture was cooled to room temperature. Quantitative ^{1}H NMR yields were determined from crude reaction mixtures, using 1,3,5-trimethoxybenzene as an internal standard prior to the purification. The mixture was diluted with H_2O (10 mL) and washed with EtOAc (3 x 10 mL) to remove the internal standard and proto-decarboxylative side-product. Then, the aqueous layer was acidified, using 1M HCl solution. The aqueous layer was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuo to give the desired product. The ^{13}C% incorporation was determined using high-resolution mass spectrometry.

For some substrates, the ^{13}C% incorporation was obtained through analysis of the corresponding carboxylic acid ester compound obtained by esterification with MeI

or BnBr. Then, MeI or BnBr (1 equiv.) was added to this solution at room temperature and stirred for 1–2 h. (Quantitative ^{1}H NMR yields were determined from crude reaction mixtures, using 1,3,5-trimethoxybenzene as an internal standard prior to the purification.) After purification through a series of extractions and column chromatography, the desired product was obtained.

General Procedure B: Potassium carboxylate salt (0.1 mmol, 1.0 equiv.) was added to a dried 4-dram vial charged with a stir bar. The vial was refilled with N_2 and evacuated under reduced pressure (3x). Under N_2 flow, using a 1 mL syringe with a 25-gauge needle, DMF or DMSO (1 mL) was added to the reaction vessel. Then, the reaction headspace is evacuated (~ 300 mtorr), via a 25-gauge needle. The remainder of the procedure is identical to the General Procedure A.

Specific Experimental Details and Product Characterization Data

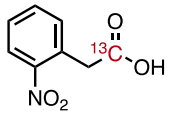


2-1 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at room temperature for 24 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 96% yield with 85% ^{13}C incorporation. *Procedure using the free acid*: Adapted from the general procedure A, using 4-cyanophenylacetic acid (0.1 mmol, 1 equiv.), K_2CO_3 (1.5 equiv.) and 18-crown-6 (1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at room temperature for 19 h. The desired product was obtained in >90% NMR yield with >95% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.63 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 3.73 (d, J = 8.5 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.9, 138.4, 132.4, 130.3, 118.5, 111.6, 40.9 (d, J = 55.1 Hz);

HRMS (ESI): calcd for C_8[^{13}C]H_7NO_2Na $[M+Na]^+$: 185.0403. Found 185.0404.

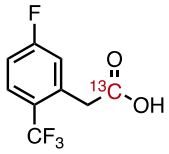


2-2 Prepared according to general procedure B from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at room temperature for 4 h. After purification through a series of extractions, as described in the general procedure B, the desired product was obtained in 87% yield with 94% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 8.14 (dd, *J* = 8.2 Hz, 1.2 Hz, 1H), 7.61 (td, *J* = 7.5 Hz, 1.2 Hz, 1H), 7.51–7.48 (m, 1H), 7.37 (d, *J* = 7.5 Hz, 1H), 4.07 (d, *J* = 8.3 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.6, 148.7, 133.9, 133.6 (d, *J* = 2.1 Hz), 129.3 (d, *J* = 2.6 Hz), 129.0, 125.5, 39.7 (d, *J* = 57.1 Hz);

HRMS (ESI): calcd for C_7[^{13}C]H_7NO_4 $[M+NH_4]^+$: 200.0743. Found 200.0743.



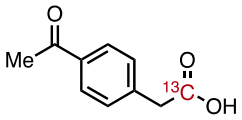
2-3 Prepared according to the general procedure B from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 60 °C for 2 h. After purification through a series of extractions, as described in the general procedure B, the desired product was obtained in 85% yield with 90% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.67 (dd, *J* = 8.7 Hz, 5.5 Hz, 1H), 7.12 dd, *J* = 9.0 Hz, 2.3 Hz, 1H), 7.09 (td, *J* = 8.2 Hz, 2.2 Hz, 1H), 3.86 (dd, *J* = 8.3 Hz, 0.8 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.0, 164.4 (d, *J* = 253.2 Hz), 134.8 (ddq, *J* = 8.7 Hz, 2.6 Hz, 1.3 Hz), 128.7 (dq, *J* = 9.8 Hz, 5.1 Hz), 125.5, 124.0 (q, *J* = 273.1 Hz), 120.0 (dd, *J* = 22.7 Hz, 1.4 Hz), 114.9 (d, *J* = 21.7 Hz), 37.9 (d, *J* = 56.0 Hz);

^{19}F NMR ($CDCl_3$, 376 MHz) δ –59.3, –107.5 (q, *J* = 7.0 Hz);

HRMS (ESI): calcd for C_8[^{13}C]$H_5F_4O_2$ $[M-H]^-$: 222.0265. Found 222.0268.

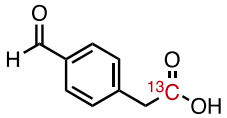


2-4 Prepared according to the general procedure B from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 80 ºC for 2 h. After purification through a series of extractions, as described in the general procedure B, the desired product was obtained in 89% yield with 95% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.94–7.92 (m, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 3.72 (d, *J* = 8.0 Hz, 2H), 2.60 (s, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 197.9, 178.4, 138.7 (d, *J* = 2.9 Hz), 136.4, 129.8 (d, *J* = 1.9 Hz), 128.9, 41.0 (d, *J* = 55.5 Hz), 26.7;

HRMS (ESI): calcd for C_9[^{13}C]$H_{10}O_3$ $[M+H]^+$: 180.0737. Found 180.0729.

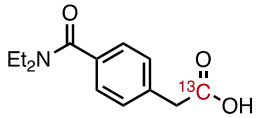


2-5 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 55 ºC for 2 h. After purification through a series of extractions, as described in the general procedure B, the desired product was obtained in 22% yield (53% NMR yield) with 66% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.01 (s, 1H), 7.86 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 3.76 (d, *J* = 7.5 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.8, 174.6, 140.1, 135.6, 130.2, 130.1, 40.8 (d, *J* = 55.4 Hz);

HRMS (ESI): calcd for C_8[^{13}C]H_9O_3 $[M+H]^+$: 166.0580. Found 166.0576.

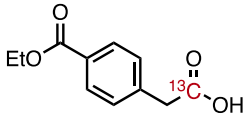


2-6 Prepared according to the general procedure B from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 80 °C for 17 h. After purification through a series of extractions, as described in the general procedure B, the desired product was obtained in 90% yield with 87% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.30–7.29 (m, 2H), 7.27–7.26 (m, 2H), 3.58 (d, *J* = 7.7 Hz, 2H), 3.55 (s, br, 2H), 3.25 (s, br, 2H), 1.24 (s, br, 3H), 1.10 (s, br, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.8, 171.9, 135.5 (d, *J* = 2.9 Hz), 135.2, 129.4 (d, *J* = 1.9 Hz), 126.7, 43.6, 41.3 (d, *J* = 55.4 Hz), 39.7, 14.3, 13.0;

HRMS (ESI): calcd for C_{12}[^{13}C]$H_{17}NO_3$ $[M+H]^+$: 237.1314. Found 237.1303.

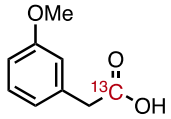


2-7 Prepared according to the general procedure B from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 70 °C for 17 h. After purification through a series of extractions, as described in the general procedure B, the desired product was obtained in 79% yield with 94% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 8.02–8.00 (m, 2H), 7.36–7.35 (m, 2H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.71 (d, *J* = 7.9 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.8, 166.5, 138.3 (d, *J* = 2.7 Hz), 130.0, 129.8, 129.6 (d, *J* = 1.9 Hz), 61.2, 41.0 (d, *J* = 55.4 Hz), 14.5;

HRMS (ESI): calcd for C_{10}[^{13}C]$H_{12}O_4$ $[M+H]^+$: 210.0842. Found 210.0836.



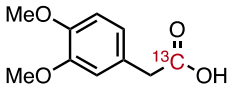
2-8 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) and 18-crown-6 (1 equiv.) in DMF and [^{13}C]CO_2.

The reaction mixture was allowed to stir at 100 ºC for 18 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 78% yield with 89% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.25–7.24 (m, 1H), 6.8–6.82 (m, 3H), 3.79 (s, 3H), 3.62 (d, *J* = 7.7 Hz, 2H);

^{13}C NMR ($CDCl_3$, 175 MHz) δ 176.9, 159.7, 134.6, 129.6, 121.7, 115.0, 112.9, 55.2, 40.9 (d, *J* = 55.1 Hz);

HRMS (ESI): calcd for $C_8[^{13}C]H_9O_3$ [M-H]$^-$: 166.0591. Found 166.0586.

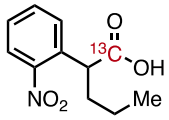


2-9 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) and 18-crown-6 (1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 115 ºC for 18 h. After purification through a series of extractions, as described in general procedure A, the desired product was obtained in 76% yield with 81% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 6.83 (s, 2H), 6.81 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.59 (d, *J* = 7.7 Hz, 2H);

^{13}C NMR ($CDCl_3$, 175 MHz) δ 177.7, 149.0, 148.4, 125.7, 121.6, 112.5, 111.3, 55.9 (2 carbon), 40.6 (d, *J* = 55.1 Hz);

HRMS (ESI): calcd for $C_9[^{13}C]H_{11}O_4$ [M-H]$^-$: 196.0696. Found 196.0691.

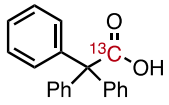


2-10 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at room temperature for 6 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 76% yield with 93% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.89 (d, *J* = 8.4 Hz, 1H), 7.6–7.54 (m, 2H), 7.43 (t, *J* =7.6 Hz, 1H), 4.24–4.20 (m, 1H), 2.17–2.13 (m, 1H), 1.86–1.83 (m, 1H), 1.40–1.25 (m, 2H), 0.92 (t, *J* = 7.5 Hz, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.8, 149.6, 133.1(2), 130.1, 128.3, 124.8, 45.9 (d, *J* = 59.4 Hz), 34.6, 20.9, 13.8;

HRMS (ESI): calcd for $C_{10}[^{13}C]H_{13}NO_4Na$ $[M+Na]^-$: 247.0770. Found 247.0769.

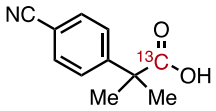


2-11 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at room temperature for 1.5 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 90% yield with 93% ^{13}C incorporation.

^{1}H NMR (DMSO-d_6, 700 MHz) δ 7.31–7.22 (m, 9H), 7.12–7.11 (m, 6H);

^{13}C NMR (DMSO-d_6, 176 MHz) δ 174.3, 143.2, 129.9, 127.6, 126.6, 66.8 (d, *J* = 54.4 Hz);

HRMS (ESI): calcd for $C_{19}[^{13}C]H_{16}O_2Na$ $[M+Na]^+$: 312.1076. Found 312.1077.

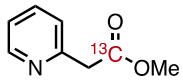


2-12 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 85 °C for 1 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 96% yield with 94% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.64 (d, *J* = 8.6 Hz, 2H), 7.51 (d, *J* = 8.6 Hz, 2H), 1.61 (s, 6H);

^{13}C NMR ($CDCl_3$, 175 MHz) δ 181.7, 149.1, 132.3, 126.9, 118.6, 111.1, 46.8 (d, *J* = 54.0 Hz), 26.1;

HRMS (ESI): calcd for $C_{10}[^{13}C]H_{11}NO_2Na$ $[M+Na]^+$: 213.0716. Found 213.0712.

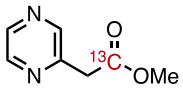


2-13 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 50 ºC for 16 h. Then, the solution was cooled down to room temperature. MeI (1 equiv.) was added to this solution and stirred for 1 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 80% yield with 48% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.57 (d, *J* = 4.8 Hz, 1H), 7.66 (td, *J* = 7.7 Hz, 1.8 Hz, 1H), 7.29 (d, *J* = 7.9 Hz, 1H), 7.19 (dd, *J* = 7.9 Hz, 15.3 Hz, 1H), 3.86 (d, *J* = 8.2 Hz, 2H), 3.73 (s, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 154.4, 149.6, 136.7, 123.9, 122.2, 52.2, 43.8 (d, *J* = 57.4 Hz);

HRMS (ESI): calcd for C_7[^{13}C]$H_{10}NO_2$ $[M+H]^+$: 153.0740. Found 153.0738.

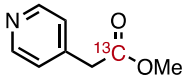


2-14 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 50 ºC for 16 h. Then, the solution was cooled down to room temperature. MeI (1 equiv.) was added to this solution and stirred for 1 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 68% yield with 29% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 8.61 (s, 1H), 8.54–8.53 (m, 1H), 8.50 (d, *J* = 2.8 Hz, 1H), 3.89 (d, *J* = 8.3 Hz, 2H), 3.75 (t, *J* = 1.8 Hz, 3H);

^{13}C NMR ($CDCl_3$, 175 MHz) δ 170.1, 150.3, 145.3, 144.2, 143.3, 52.4, 41.1 (d, *J* = 57.8 Hz);

HRMS (ESI): calcd for C_6[^{13}C]$H_9N_2O_2$ $[M+H]^+$: 154.0692. Found 154.0696.

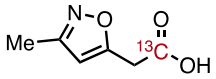


2-15 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at room temperature for 15 h. Then, MeI (1 equiv.) was added to this solution and stirred for 1 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 69% yield with 41% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 8.56 (dd, *J* = 4.5 Hz, 1.7 Hz, 2H), 7.22 (dd, *J* = 4.5 Hz, 1.7 Hz, 2H), 3.72 (t, *J* = 1.4 Hz, 3H), 3.63 (d, *J* = 7.7 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.5, 150.0, 142.7, 124.5, 52.3, 40.5 (d, *J* = 57.8 Hz);

HRMS (ESI): calcd for C_7[^{13}C]$H_{10}NO_2$ $[M+H]^+$: 153.0740. Found 153.0740.

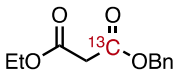


2-16 Prepared according to the general procedure B from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 40 °C for 24 h. After purification through a series of extractions, as described in the general procedure B, the desired product was obtained in 49% yield (99% NMR yield) with 56% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 6.13 (s, 1H), 3.84 (d, *J* = 7.9 Hz, 2H), 2.29 (s, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.6, 164.3, 160.3, 104.6, 32.5 (d, *J* = 58.8 Hz), 11.6;

HRMS (ESI): calcd for C_5[^{13}C]H_7NO_3 $[M+H]^+$: 143.0529. Found 143.0530.

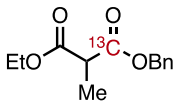


2-17 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 40 °C for 21 h. Then, the solution was cooled down to room temperature. BnBr (1 equiv.) was added to this solution and stirred for 2 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 82% yield with 37% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.38–7.33 (m, 5H), 5.19 (t, *J* = 1.8 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.42 (d, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H);

^{13}C NMR ($CDCl_3$, 175 MHz) δ 166.5, 166.4, 135.3, 128.6, 128.4, 128.3, 67.2, 61.6, 41.6 (d, *J* = 59.2 Hz), 14.0;

HRMS (ESI): calcd for $C_{11}[^{13}C]H_{14}O_4Na$ $[M+Na]^+$: 246.0818. Found 246.0818.

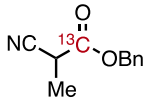


2-18 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 60 ºC for 21 h. Then, the mixture was cooled down to room temperature. BnBr (1 equiv.) was added to this solution and stirred for 2 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 75% yield with 89% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.31 (m, 5H), 5.22–5.15 (m, 2H), 4.21– 4.12 (m, 2H), 3.5 –3.45 (m, 1H), 1.45–1.43 (m, 3H), 1.21 (t, *J* = 7.2 Hz, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2, 170.0, 135.6, 128.6, 128.3, 128.1, 70.0, 61.4, 46.3 (d, *J* = 58.1 Hz), 14.0, 13.6;

HRMS (ESI): calcd for $C_{12}[^{13}C]H_{16}O_4Na$ $[M+Na]^+$: 260.0974. Found 260.0974.

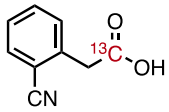


2-19 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 60 ºC for 18 h. Then, the mixture was cooled down to room temperature. BnBr (1 equiv.) was added to this solution and stirred for 2 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 82% yield with 67% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.39–7.36 (m, 5H), 5.26–5.22 (m, 2H), 3.61–3.56 (m, 1H), 1.61–1.60 (m, 3H);

^{13}C NMR ($CDCl_3$, 175 MHz) δ 166.4, 134.5, 128.8, 128.7, 128.3, 117.1, 68.3, 31.6 (d, J = 59.8 Hz), 15.2;

HRMS (ESI): calcd for $C_{10}[^{13}C]H_{11}NO_2Na$ $[M+Na]^+$: 213.0716. Found 213.0717.

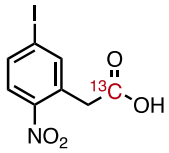


2-20 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 80 ºC for 19 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 81% yield with 81% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68–7.66 (m, 1H), 7.57 (td, J = 8.2 Hz, 1.3 Hz, 1H), 7.38–7.43 (m, 2H), 3.92 (d, J = 7.4 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.2, 137.1, 132.9, 132.8, 130.7, 127.9, 117.4, 113.5, 39.3 (d, J = 62.6 Hz);

HRMS (APPI): calcd for $C_8[^{13}C]H_6NO_2$ $[M-H]^-$: 161.0438. Found 161.0434.

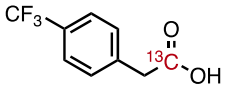


2-21 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at room temperature for 6 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 77% yield with 63% ^{13}C incorporation.

^{1}H NMR (CD_3OD, 400 MHz) δ 7.91–7.89 (m, 2H), 7.8–7.81 (m, 1H), 3.99 (d, J = 8.1 Hz, 2H);

^{13}C NMR (CD_3OD, 100 MHz) δ 173.3, 150.0, 143.5, 138.9, 133.5, 127.3, 101.6, 39.8 (d, J = 55.3 Hz);

HRMS (APPI): calcd for $C_7[^{13}C]H_6INO_4$ $[M]^-$: 307.9381. Found 307.9377.



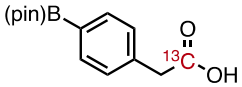
2-22 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 90 °C for 26 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 83% yield with 92% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.60 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 3.72 (d, *J* = 7.7 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.0, 137.3, 130.3, 129.9 (t, *J* = 15.4 Hz), 125.7, 124.1 (q, *J* = 271.8 Hz), 40.7 (d, *J* = 55.8 Hz);

^{19}F NMR ($CDCl_3$, 376 MHz) δ –62.6;

HRMS (APPI): calcd for C_8[^{13}C]$H_6F_3O_2$ $[M-H]^-$: 204.0359. Found 204.0356.

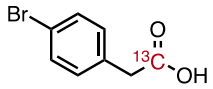


2-23 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 100 °C for 5 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 73% yield with 93% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.78 (d, *J* = 7.9 Hz, 2H), 7.29 (d, *J* = 7.9 Hz, 2H), 3.66 (d, *J* = 7.9 Hz, 2H), 1.36 (s, 12H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.2, 136.4, 135.2, 128.8, 83.8, 41.1 (d, *J* = 55.2 Hz), 24.9 (the quaternary carbon attached to boron was not observable);

HRMS (ESI): calcd for C_{13}[^{13}C]H_{23}[^{11}B]NO_4 $[M+NH_4]^+$: 281.1748. Found 281.1748.

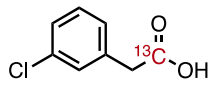


2-24 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 110 ºC for 14 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 77% yield with 95% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.46 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 8.3 Hz, 2H), 3.61 (d, *J* = 8.7 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.4, 158.8., 130.4, 125.3, 114.1, 40.1 (d, *J* = 55.9 Hz);

HRMS (APPI): calcd for C_7[^{13}C]H_6BrO_2 [M-H]$^-$: 213.9590. Found 213.9590.

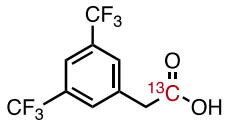


2-25 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 110 ºC for 9 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 77% yield with 87% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31–7.28 (m, 3H), 7.20–7.18 (m, 1H), 3.65 (d, *J* = 7.8 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.7, 135.1, 134.5, 129.9, 129.8, 129.6, 127.6, 40.6 (d, *J* = 56.2 Hz);

HRMS (APPI): calcd for C_7[^{13}C]H_6ClO_2 [M-H]$^-$: 170.0095. Found 170.0093.



2-26 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 80 ºC for 19 h. After purification through a series of extractions, as

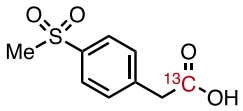
described in the general procedure A, the desired product was obtained in 85% yield with 84% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82 (s, 1H), 7.75 (s, 2H), 3.81 (d, J = 7.9 Hz, 2H);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.3, 135.5, 132.1 (q, J = 33.2 Hz), 129.8, 123.2 (q J = 272.0 Hz), 121.6 (q, J = 3.8 Hz), 40.3 (d, J = 58.8 Hz);

^{19}F NMR ($CDCl_3$, 376 MHz) δ –62.9;

HRMS (APPI): calcd for $C_9[^{13}C]H_5F_6O_2$ $[M-H]^-$: 272.0233. Found 272.0239.

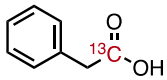


2-27 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 70 °C for 5 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 89% yield with 87% ^{13}C incorporation.

^{1}H NMR (CD_3OD, 600 MHz) δ 8.1 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 3.95 (d, J = 7.9 Hz, 2H), 3.10 (s, 3H);

^{13}C NMR (CD_3OD, 125 MHz) δ 174.1, 142.7, 140.6, 131.6, 128.5, 44.4, 41.8 (d, J = 58.8 Hz);

HRMS (ESI): calcd for $C_8[^{13}C]H_{10}O_4SNa$ $[M+Na]^+$: 238.0226. Found 238.0225.

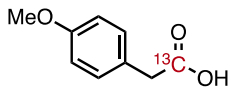


2-28 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 120 °C for 47 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 83% yield with 94% ^{13}C incorporation. When the reaction mixture was stirred at 100 °C for 47 h in the presence of 18-crown-6 (1 equiv.), the desired product was obtained in 92% NMR yield with 81% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35–7.27 (m, 5H), 3.66 (d, J = 7.7 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.4, 133.3, 129.4, 128.7, 127.4, 41.2 (d, *J* = 54.0 Hz);

HRMS (APPI): calcd for $C_7[^{13}C]H_7O_2$ $[M-H]^-$: 136.0485. Found 136.0485.

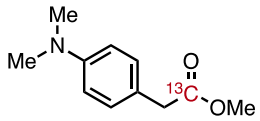


2-29 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 140 ºC for 21 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 84% yield with 72% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.20 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 3.79 (s, 3H) 3.59 (d, *J* = 7.8 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.8, 132.3, 131.8, 131.2, 121.6, 55.2, 40.4 (d, *J* = 55.3 Hz);

HRMS (APPI): calcd for $C_8[^{13}C]H_9O_3$ $[M-H]^-$: 166.0591. Found 166.0590.

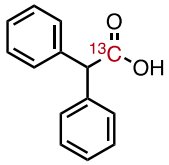


2-30 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 140 ºC for 25 h. Then, MeI (1 equiv.) was added to this solution at room temperature and stirred for 1 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 68% yield with 34% ^{13}C incorporation. When the reaction mixture was stirred at 130 ºC for 25 h in the presence of 18-crown-6 (1 equiv.), the desired product was obtained in 72% NMR yield with 89% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.15 (d, *J* = 8.7 Hz, 2H), 6.69 (d, *J* = 8.7 Hz, 2H), 3.67 (t, *J* = 1.8 Hz, 3H), 3.53 (d, *J* = 7.9 Hz, 2H), 2.93 (s, 6H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.8, 149.8, 129.9, 121.8, 112.8, 51.9, 40.8, 40.3 (d, *J* = 60.0 Hz);

HRMS (APPI): calcd for $C_{10}[^{13}C]H_{14}NO_2$ $[M-H]^-$: 193.1064. Found 193.1061.

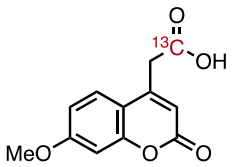


2-31 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 90 ºC for 2 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 83% yield with 92% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.34–7.27 (m, 10H), 5.10 (d, *J* = 8.5 Hz, 1H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.1, 138.0, 128.8, 128.7, 127.6, 57.0 (d, *J* = 55.7 Hz);ß

HRMS (ESI): calcd for $C_{13}[^{13}C]H_{12}O_2Na$ $[M+Na]^+$: 236.0763. Found 236.0762.

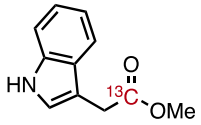


2-32 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at room temperature for 20 min. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 83% yield with 27% ^{13}C incorporation.

^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.62 (d, *J* = 8.8 Hz, 1H), 7.01 (d, *J* = 2.6 Hz, 1H), 6.96 (dd, *J* = 8.8 Hz, 2.6 Hz, 1H), 6.29 (s, 1H), 3.86 (d, *J* = 7.2 Hz, 2H), 3.85 (s, 3H);

^{13}C NMR (DMSO-d_6, 150 MHz) δ 171.0, 162.8, 160.5, 155.4, 150.5, 127.0, 113.3, 112.9, 112.6, 101.3, 56.4, 37.7;

HRMS (ESI): calcd for $C_{11}[^{13}C]H_{10}O_5Na$ $[M+Na]^+$: 257.0464. Found 257.0462.

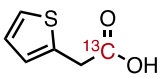


2-33 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 140 ºC for 60 h. Then, MeI (1 equiv.) was added to this solution at room temperature and stirring was continued for 1 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 95% yield with 18% ^{13}C incorporation. When the reaction mixture was stirred at 130 ºC for 25 h in the presence of 18-crown-6 (1 equiv.), the desired product was obtained in 64% NMR yield with 65% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.07 (s, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.13–7.22 (m, 3H), 3.80 (d, *J* = 7.7 Hz, 2H), 3.70 (t, *J* = 2.1 Hz, 3H);

^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.5, 136.2, 127.3, 123.1, 122.3, 119.8, 118.9, 111.2, 108.6, 52.0, 31.2;

HRMS (ESI): calcd for C_{10}[^{13}C]$H_{11}NO_2Na$ $[M+Na]^-$: 213.0716. Found 213.0716.

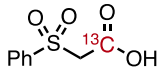


2-34 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at 100 ºC for 20 h. After purification through a series of extractions, as described in the General Procedure A, the desired product was obtained in 70% NMR yield with 89% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.24 (dd, *J* = 4.7 Hz, 1.9 Hz, 1H), 6.98–6.97 (m, 2H), 3.88 (d, *J* = 7.8 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.9, 134.1, 127.3, 126.9, 125.5, 35.0 (d, *J* = 55.1 Hz);

HRMS (ESI): calcd for C_5[^{13}C]H_5SO_2 $[M-H]^-$: 142.0049. Found 142.0050.

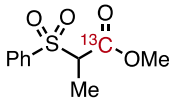


2-35 Prepared according to the general procedure B from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at room temperature for 2 h. After purification through a series of extractions, as described in the general procedure B, the desired product was obtained in 74% yield with 67% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.98–7.96 (m, 2H), 7.71 (tt, *J* = 7.5 Hz, 1.4 Hz, 1H), 7.62–7.58 (m, 2H), 4.15 (d, *J* = 6.3 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.9, 138.6, 134.7, 129.6, 128.7, 60.7 (d, *J* = 58.2 Hz);

HRMS (ESI): calcd for C_7[^{13}C]H_8O_4S $[M+Na]^+$: 224.0066. Found 224.0070.

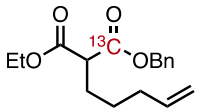


2-36 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at room temperature for 26 h. Then, MeI (1 equiv.) was added to this solution and stirred for 1 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 84% yield with 80% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.89–7.87 (m, 2H), 7.70–7.67 (m, 1H), 7.59–7.56 (m, 2H), 4.10–4.03 (m, 1H), 3.68 (t, *J* = 1.8 Hz, 3H), 1.58–1.55 (m, 3H);

^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.7, 136.9, 134.3, 129.4, 129.1, 65.4 (d, *J* = 60.0 Hz), 53.0, 11.9;

HRMS (ESI): calcd for C_9[^{13}C]$H_{12}SO_4Na$ $[M+Na]^+$: 252.0382. Found 252.0381.



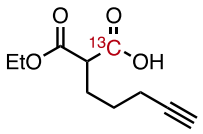
2-37 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was

allowed to stir at 60 ºC for 22 h. Then, the mixture was cooled down to room temperature. BnBr (1 equiv.) was added to this solution and stirred for 2 h. After purification through a series of extractions and column chromatography, the desired product was obtained in 80% yield with 94% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.36–7.32 (m, 5H), 5.78–5.72 (m, 1H), 5.2–5.15 (m, 2H), 5.02–4.94 (m, 2H), 4.16 (q, *J* = 7.8 Hz, 2H), 3.39–3.36 (m, 1H), 2.06 (q, *J* = 6.4 Hz, 2H), 1.94–1.90 (m, 2H), 1.44–1.39 (m, 2H), 1.20 (t, *J* = 7.2 Hz, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.3(2), 137.9, 135.6, 128.6, 128.4, 128.2, 115.1, 67.0, 61.4, 51.9 (d, *J* = 57.8 Hz), 33.3, 28.2, 26.6, 14.1;

HRMS (ESI): calcd for $C_{16}[^{13}C]H_{22}O_4Na$ $[M+Na]^+$: 314.1444. Found 314.1442.

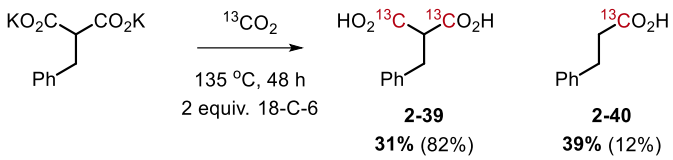


2-38 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was stirred at 60 ºC for 22 h. After purification through a series of extractions, as described in the general procedure A, the desired product was obtained in 71% yield with 87% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.23 (q, *J* = 7.2 Hz, 2H), 3.43–3.39 (m, 1H), 2.24 (td, *J* = 6.9, 2.5 Hz, 2H), 2.06–2.02 (m, 2H), 1.96 (t, *J* = 2.7 Hz, 1H), 1.61 (q, *J* = 8.1 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 3H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.2, 169.3, 83.3, 69.1, 61.9, 51.2 (d, *J* = 55.5 Hz), 27.9, 26.1, 18.1, 14.1;

HRMS (ESI): calcd for $C_9[^{13}C]H_{14}O_4Na$ $[M+Na]^+$: 222.0818. Found 222.0819.



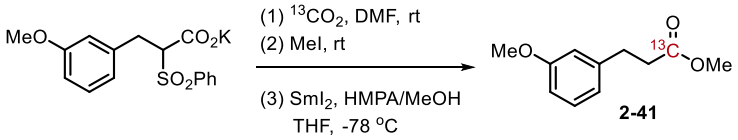
2-39 and **2-40** Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) and 18-crown-6 (2 equiv.) in DMF

and [^{13}C]CO_2. The reaction mixture was allowed to stir at 135 ºC for 48 h. After purification through a series of extractions, as described in the General Procedure A, the desired products **2-39** and **2-40** were obtained [**2-39**: 82% NMR yield with 31% ^{13}C incorporation (19% *di*-^{13}C incorporation and 12% *mono*-^{13}C incorporation); **2-40**: 12% NMR yield with 39% ^{13}C incorporation].

^{1}H NMR (CD_3OD, 500 MHz) δ Product **2-39**: 7.46–7.34 (m, 5H), 3.83–3.77 (m, 1H), 3.33 (d, *J* = 7.7 Hz, 2H); Product **2-40**: 7.46–7.34 (m, 5H), 3.22–3.06 (m, 2H), 2.8–2.74 (m, 2H);

^{13}C NMR (CD_3OD, 175 MHz) δ Product **2-39**: 172.7, 139.9, 129.9, 129.5, 127.7, 54.8 (m), 35.3; Product **2-40**: Only see the incorporated carbon from carboxylic acid due to trace of product **2-40**, δ: 176.8 (^{13}C);

HRMS (ESI): 2-39: *di*-^{13}C incorporation product: calcd for $C_8[^{13}C]_2H_9O_4$ $[M-H]^-$: 195.0573. Found 195.0570. mono-^{13}C incorporation product: calcd for $C_9[^{13}C]H_9O_4$ $[M-H]^-$: 194.0540. Found 194.0539.



2-41 Prepared according to the general procedure A from the corresponding carboxylate salt (0.2 mmol, 1 equiv.) in DMF and [^{13}C]CO_2. The reaction mixture was allowed to stir at room temperature for 19 h. Then, MeI (1 equiv.) was added to this solution and stirred for 1 h. After purification through a series of extractions and column chromatography, ^{13}C-labeled methyl 2-benzenesulfonyl-3-phenylpropionate was obtained.

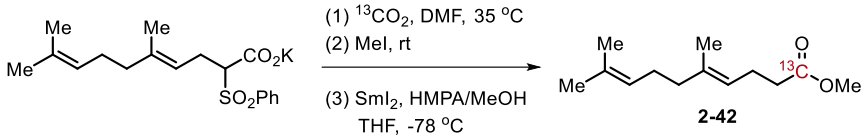
Adapted from the literature,[133] a solution of SmI_2 (6.25 equiv.; 0.1 M solution in THF) was placed under N_2 atmosphere, and then HMPA (8.0 equiv.) and MeOH (50 equiv.) were added. ^{13}C-labeled methyl 2-benzenesulfonyl-3-phenylpropionate (1.0 equiv.) in THF (0.1 M) was added at −78 °C. (*If the typical deep blue color of SmI_2 faded upon the sulfone solution addition, additional SmI_2 was added until the deep blue color persisted.*) The resulting mixture was stirred at −78 °C for an additional 5 h. The saturated aq. NH_4Cl was added, and the aqueous layer was extracted with EtOAc (x3).

The combined organic layers were washed with saturated aq. $Na_2S_2O_3$, brine, dried over $MgSO_4$ and evaporated under reduced pressure to give the crude mixture. Chromatography on silica gel (10:1 Hexane:EtOAc) gave the title compound **2-41** (the overall yield of 64% with 66% ^{13}C incorporation).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22–7.19 (m, 1H), 6.79 (d, *J* = 7.5 Hz, 1H), 6.7– 6.74 (m, 2H), 3.79 (s, 3H), 3.67 (t, *J* = 1.9 Hz, 3H), 2.94–2.91 (m, 2H), 2.65–2.61(m, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 159.7, 142.1, 129.5, 120.6, 114.1, 111.6, 55.2, 51.6, 35.6 (d, *J* = 57.3 Hz), 31.1;

HRMS (ESI): calcd for $C_{10}[^{13}C]H_{14}O_3$ $[M]^+$: 195.0977. Found 195.0977.

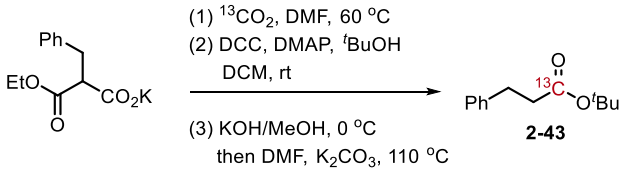


2-42 Prepared according to the general procedure A from the corresponding carboxylate salt (0.2 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 35 °C for 25 h. Then, MeI (1 equiv.) was added to this solution and stirred for 1 h at rt. After purification through a series of extractions and column chromatography, ^{13}C-labeled sufone was obtained. Adapted from the literature,[132] a solution of SmI_2 (6.25 equiv.; 0.1 M solution in THF) was placed under N_2 atmosphere, then HMPA (8.0 equiv.) and MeOH (50 equiv.) were added. ^{13}C-labeled sulfone (1.0 equiv.) in THF (0.1 M) was added at −78 °C. (*If the typical deep blue color of SmI_2 faded upon the sulfone solution addition, additional SmI_2 was added until the deep blue color persisted.*) The resulting mixture was stirred at −78 °C for an additional 5 h. The saturated aq. NH_4Cl was added, and the aqueous layer was extracted with EtOAc (x3). The combined organic layers were washed with saturated aq. $Na_2S_2O_3$, and brine, dried over $MgSO_4$ and evaporated under reduced pressure to give the crude mixture. Chromatography on silica gel (20:1 Hexane:EtOAc) gave the title compound **2-42** (the overall yield of 74% with 65% ^{13}C incorporation).

^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.11–5.05 (m, 2H), 3.66 (t, *J* = 1.8 Hz, 3H), 2.35–2.30 (m, 4H), 2.07–2.03 (m, 2H), 1.98–1.94 (m, 2H), 1.67 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H);

^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.9, 136.8, 131.5, 124.2, 122.4, 51.5, 39.7, 34.3 (d, *J* = 57.6 Hz), 26.7, 25.7, 23.6, 17.7, 16.1;

HRMS (ESI): calcd for $C_9[^{13}C]H_{15}O_2$ $[M]^+$: 168.1106. Found 168.1105.



2-43 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at 60 °C for 6 h. After purification through a series of extractions, as described in the General Procedure A, ^{13}C-labeled 2-benzyl-3-ethoxy-3-oxopropanoic acid was obtained. When the reaction mixture was stirred at 40 °C for 1 h in the presence of 18-crown-6 (1 equiv.), the desired product was obtained in 95% yield with >95% ^{13}C incorporation.

Adapted from the literature,[134] a solution of the above ^{13}C-labeled carboxylic acid in DCM (0.3 mL) was added to a solution of ^{t}BuOH (1.25 equiv.) and DMAP (0.03 equiv.) in DCM (0.15 mL) at 0 °C. A solution of N, N'-dicyclohexylcarbodiimide (1.3 equiv.) in DCM (0.1 mL) was added. The mixture was stirred overnight at room temperature, and the precipitate formed was filtered off through celite with Et_2O. After removing the solvent under vacuum, the residue was chromatographed on silica gel (10:1 Hexane:EtOAc) to afford ^{13}C-labeled 1-*tert*-butyl 3-ethyl 2-benzylmalonate.

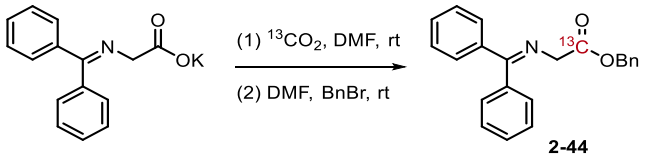
Adapted from the literature,[135] KOH (1M, 2.0 equiv.) in MeOH solution was added into ^{13}C-labeled 1-*tert*-butyl 3-ethyl 2-benzylmalonate at 0 °C. The reaction was stirred for 3 h, and then the solvent was removed in vacuo. The resulting yellowish oils were dissolved further in saturated $NaHCO_3$ and washed with Et_2O to remove traces of unreacted starting materials. Then, the aqueous layer was acidified with conc. HCl until approx. pH = 2 and extracted with EtOAc (x2). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give ^{13}C-labeled 2-benzyl-3-(*tert*-butoxy)-3-oxopropanoic acid. Then, to a solution of ^{13}C-labeled 2-benzyl-3-(*tert*-butoxy)-3-oxopropanoic acid in DMF (0.5 mL) was added

K_2CO_3 (1.0 equiv.) and stirred at 110 °C for 14 h. The solution was extracted with EtOAc (x3), washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel (20:1 hexane: EtOAc) to afford the title compound **2-43** (the overall yield of 61% with 77% ^{13}C incorporation).

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.29–7.27 (m, 2H), 7.21–7.18 (m, 3H), 2.92–2.89 (tt, *J* = 7.5 Hz, 2.2 Hz, 2H), 2.92– .89 (m, 2H), 1.42 (s, 9H);

^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.4, 140.9, 128.5, 128.4, 126.2, 80.4, 37.2 (d, *J* = 57.1 Hz), 31.2, 28.2;

HRMS (ESI): calcd for $C_{12}[^{13}C]H_{18}O_2Na$ $[M+Na]^+$: 230.1233. Found 230.1236.



2-44

2-44 Prepared according to the general procedure A from the corresponding carboxylate salt (0.1 mmol, 1 equiv.) in DMF and $[^{13}C]CO_2$. The reaction mixture was allowed to stir at rt for 24 h. The ^{13}C isotope incorporation was determined to be 93% through analysis of high-resolution mass spectrum of the corresponding carboxylic acid. Then, BnBr (1 equiv.) was added to this solution and stirred for 2 h at room temperature. The mixture was diluted in DCM (10 mL) and sat. $NaHCO_3$ solution (5 mL). The aqueous layer was extracted with DCM (3 x 10 mL), and the combined organic layers were washed with sat. $NaHCO_3$ solution (5 mL), dried over anhydrous $NaSO_4$, filtered, and concentrated in vacuo to give the desired product **61** in 76% yield with 93% ^{13}C incorporation.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.66 (d, *J* = 7.6 Hz, 2H), 7.46–7.32 (m, 11H), 7.16 (dd, *J* = 7.7 Hz, 3.7 Hz, 2H), 5.19 (d, *J* = 2.7 Hz, 2H), 4.26 (d, *J* = 7.0 Hz, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.1 (q), 170.5, 139.3, 136.0, 135.8, 130.5, 128.8, 128.7 (2), 128.6, 128.3, 128.2, 128.1, 127.7, 65.6, 55.7 (d, *J* = 63.4 Hz);

HRMS (ESI): calcd for $C_{12}[^{13}C]H_{20}NO_2$ $[M+H]^+$: 331.1522. Found 331.1520.

CHAPTER 3

Synthesis of Isotopically-labeled Racemic α-Amino Acids Using Achiral Aldehyde Catalysis in Conjuction with Isotopically-labeled CO_2

3.1 Introduction

α-Amino acids are a family of organic compounds containing carboxyl and amine functional groups, which are a major component of protein scaffolds and hence of biological structures. α-Amino acids are a key building block of life and are found increasingly in clinical candidates (for example, antivirals ledipasvir, cobicistat, voxilaprevir, and anti-cancer drugs methotrexate, bortezomib, carfilzomib, alpelisib) (**Fig. 3–1**).[136-139]

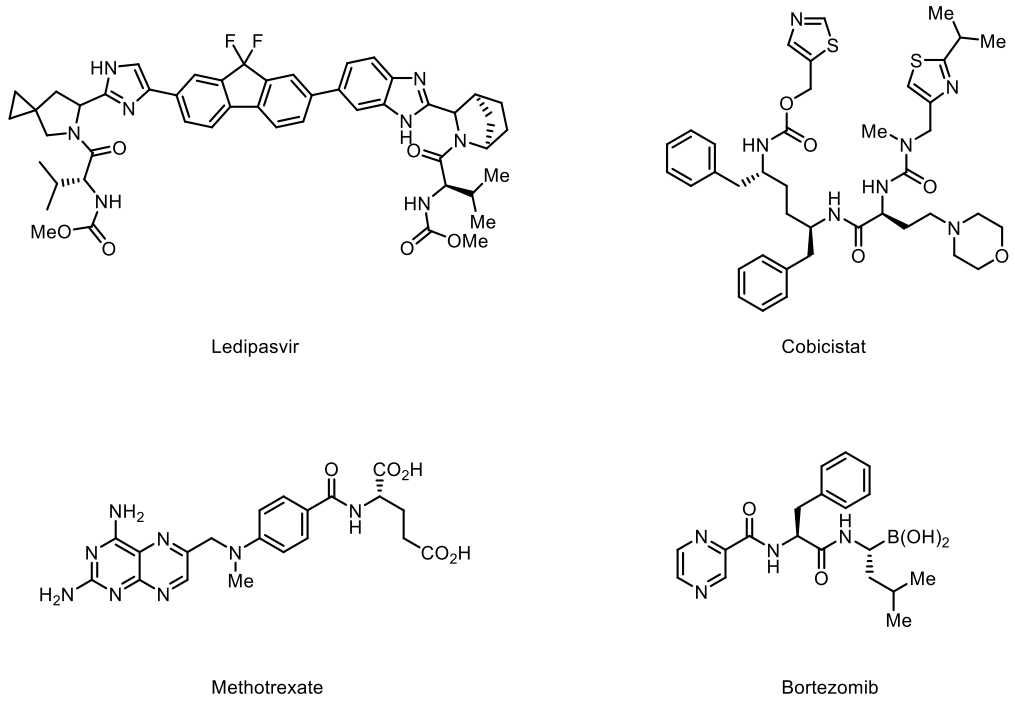


Fig. 3–1. α-Amino acids in clinical candidates.

Isotopically labeled α-amino acids and their derivatives have widespread use in structural and mechanistic biochemistry,[140] quantitative proteomics,[141] absorption

distribution metabolism and excretion (ADME) profiling,[142-143] and as imaging agents in positron emission tomography (PET) techniques.[144-146]

The incorporation of $^{13/14}C$ into the α-amino acids can be done by using classical methods. General approaches include the cyanation of electrophiles with [^{13}C]CN, followed by hydrolysis (**Fig. 3–2a**),[147-149] carboxylation of organometallic with [^{13}C]CO_2 (**Fig. 3–2b**),[150-151] the use of C-labeled acetate as a precursor (**Fig. 3–2c**),[152-153] and alkylations with ^{13}C-labeled electrophiles (**Fig. 3–2d**).[154] These methods can be utilized to prepare enantioenriched isotopically labeled α-amino acids via enzymatic approach after the insertion of labeled carbon. The enzymatic approach includes enzymatic kinetic resolution, such as acylase, as shown in (**Fig. 3–2a**),[147-149] and the use of lyases for stereoselective C–N bond formation, as shown in (**Fig. 3–2c**).[152-153] It also can be done by using chiral auxiliaries for α-alkylation or α-amination, as shown in (**Fig. 3–2d**),[154] and enantioselective hydrogenation of enamines, as shown in (**Fig. 3–2e**).[155]

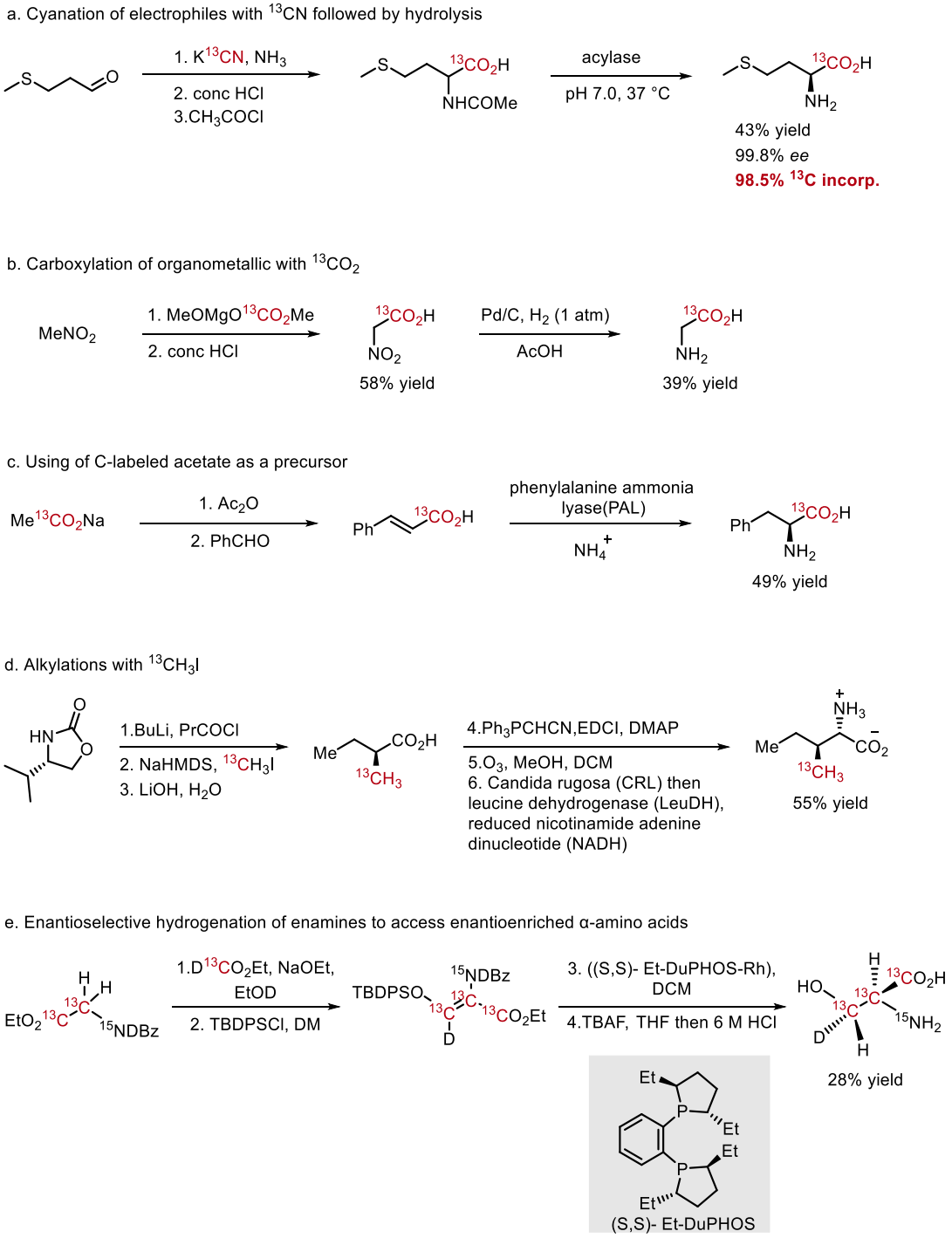


Fig. 3–2. Classical methods for synthesis of ^{13}C-labeled-α-amino acids.

The short half-life of ^{11}C (20 min) makes the multi-step preparation of ^{11}C-α-amino acids targets needed for PET problematic. Current approaches are restricted to cyanation/hydrolysis reactions. using [^{11}C]CN$^-$ (**Fig. 3–3a**),[156] Another approach

includes the use of [^{11}C]CH_3I; an example of this is methylation of methionine with $^{11}CH_3I$ to get [S-methyl-^{11}C]methionine (**Fig. 3–3b**).[157] [C_1-^{11}C]glutamine or glutamate can be prepared by conjugate additions to ^{11}C-acrylates (**Fig. 3–3c**).[158] All these methods occur with low to moderate radiochemical yields and require time-consuming, multi-step approaches.

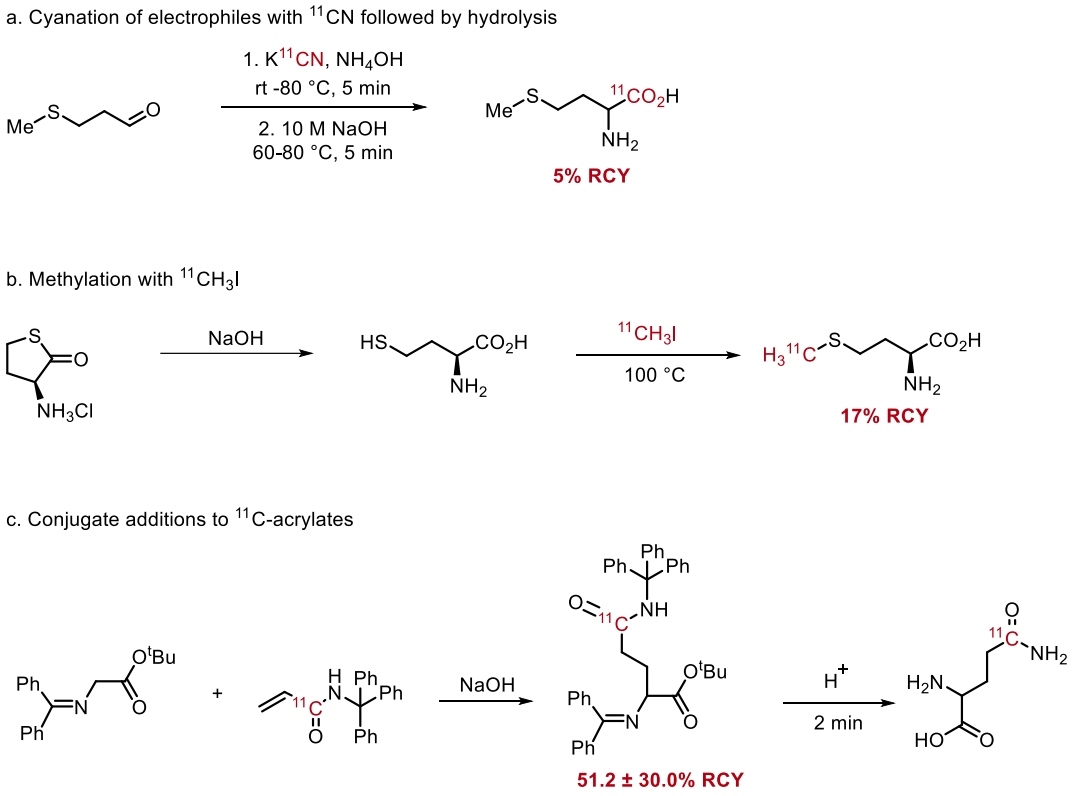


Fig. 3–3. Classical methods for synthesis of ^{11}C-α-amino acids.

Nature catalyzes CO_2 extrusion from α-amino acids with decarboxylases, which convert α-amino acids to Schiff bases (imines) via condensation with pyridoxal phosphate (**Fig. 3–4**). After condensation between an α-amino acid and the aldehyde unit of pyridoxal phosphate, decarboxylation leads to an aza-allyl quinonoid intermediate, which subsequently is protonated and hydrolyzed to give an amine product.[91,159] The small molecule catalysis of α-amino acids decarboxylation is known but occurs only at high temperatures (150 ºC).[160]

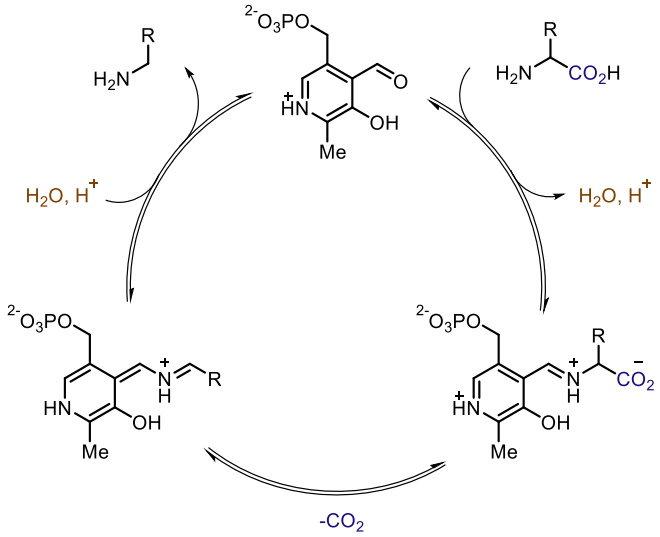


Fig. 3–4. Mechanism for pyridoxal phosphate catalyzed α-amino acid decarboxylation.

α-Amino acids lack the required anion stabilizing ability to undergo decarboxylation under normal laboratory conditions, and their spontaneous decarboxylation is exceptionally slow.[161] The established methods for carboxylate exchange of α-amino acids include chemical activation-decarboxylation-metalation-carboxylation sequences mediated by transition metals. Baran et al. reported using *N*-hydroxyphthalimide derivatives in the presence of Ni-promotors to generate [C_5-^{13}C] glutamic acid, however, C1-carboxylate and amino group protection is required, and the process is not general for other amino acids (**Fig. 3–5a**).[113] Also, carboxylate exchange of α-amino acids can be carried out under photochemical conditions. For example, certain *N*-protected cyclic α-amino acids can undergo oxidation-induced decarboxylation and subsequent recapture of CO_2 after reactions with activated alkenes to form γ-amino acids (**Fig. 3–5b**).[162]

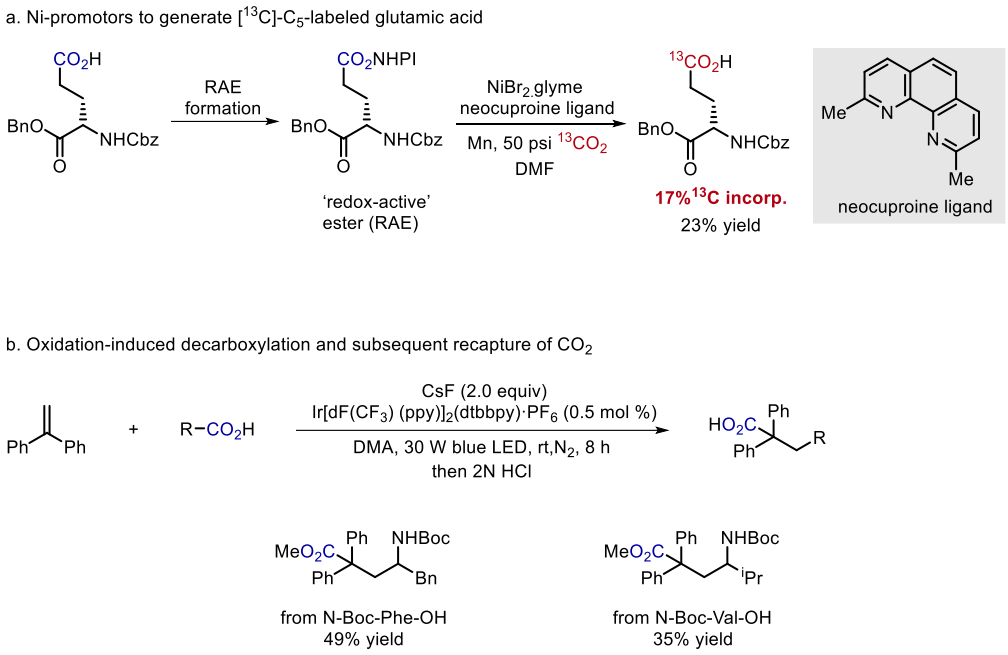


Fig. 3–5. Literature methods for carboxylate exchange of α-amino acids.

Knowing that nature can catalyze CO_2 extrusion from α-amino acids with decarboxylases, we questioned whether we could use an aldehyde catalyst to develop a reversible carboxylate exchange reaction in α-amino acids. Potentially, this would help with the preparation of carbon-11 labeled amino acids that are needed in PET techniques. Section 3.2 describes the development of the reversible decarboxylation of α-amino acids with isotopically labeled CO_2 in the presence of achiral aldehyde catalyst.

3.2 Development of Aldehyde-Catalyzed Carboxylate Exchange in α-Amino Acids with Isotopically-labeled CO_2

3.2.1 Discovery and Optimization of the Methodology

α-Amino acid derived imino carboxylic acids are known to undergo α-H/D exchange in the presence of D_2O.[163] In addition to that, in concert with Audisio and co-workers, we established that certain electronically-stabilized carboxylic acids, like aryl acetates and malonic half-esters, undergo reversible decarboxylation in polar aprotic solvents by thermal[123,164] or photochemical methods.[165-166] In particular, we showed in a previous project[164] that the potassium salt of diphenylmethylidene glycine can undergo

reversible exchange at room temperature, which serves as a starting point for this project. Given the propensity of α-amino acid derived imino carboxylic acids to undergo α-H/D exchange in the presence of D_2O[163] and the potential for decarboxylation to be reversible in a range of contexts,[123,164-166] we viewed this reaction manifold as an opportunity to develop methods for the direct C1-labeling of α-amino acids using [^{13}C]CO_2.

Initially, we tried to mimic the conditions for the potassium salt of diphenylmethylidene glycine (**Fig. 3–6a)** by preparing the imine carboxylate derivatives for other amino acids, like alanine, and then subjected them to the exchange conditions from our previous project.[164] However, we observed a low chemical yield and moderate incorporation. The low chemical yield might be due to other side products which include protodecarboxylation and trapped aldehyde products (**Fig. 3–6b**).

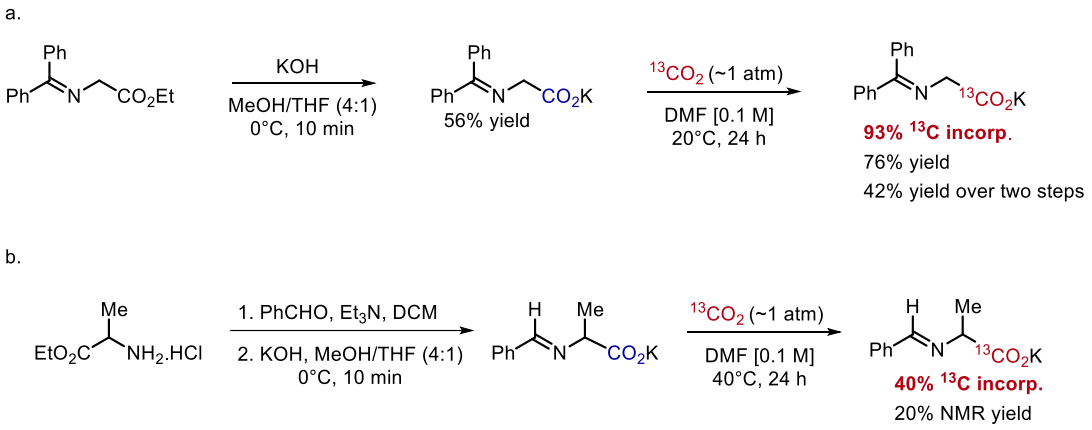
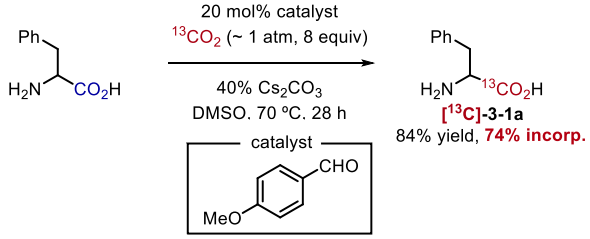


Fig. 3–6. Reversible decarboxylation of α-amino acid derivatives.

After that, we examined the possibility of using an aldehyde catalyst and α-amino acid in situ with conjunction of [^{13}C]CO_2 to develop a one-pot strategy for the labeling of α-amino acids. After a wide range of screening of aldehyde catalysts, bases, and solvents, we found that in the presence of 20 mol% 4-anisaldehyde and 40 mol% Cs_2CO_3 in DMSO at 70 ºC, C1-exchange in phenylalanine to generate (±)-[C$_1$-^{13}C]phenylalanine is observed with 75% ^{13}C incorporation and 84% yield when ~8 equivalents of [^{13}C]CO_2 are supplied. **Fig. 3–7** provides an overview of experimental

parameters important to observing a productive α-amino acid carboxylate exchange with externally supplied [^{13}C]CO_2. Other polar aprotic solvents (DMF or DMA) can be used, but the extent of incorporation is decreased. The reaction does not occur in MeOH or H_2O, and unlabeled substrate is recovered quantitatively. Trace carboxylate exchange is detected at room temperature with near-quantitative recovery of substrate, while at 130 ºC, high incorporation and yields are observed after 30 min, using 75% catalyst (60% incorporation, 80% yield). When approximately one equivalent of [^{13}C]CO_2 is supplied, exchange equilibrium nearly is achieved with a moderate reduction in yield (40% incorporation, 65% yield). Catalyst loadings as low as 5 mol% can be used in combination with higher reaction temperatures (90 ºC). Without catalyst, no isotope exchange is observed up to 160 ºC.



variation to standard conditions	^{13}C inc. (%)	y (%)
no change	74	84
DMF instead of DMSO	26	90
DMA instead of DMSO	27	83
MeOH or H_2O instead of DMSO	<2	>95
rt instead of 70 °C	5	95
50 °C, 75% cat. (24 h)	76	69
130 °C, 75% cat. (0.5 h)	60	80
160 ºC no catalyst	<2	<2
5% cat instead of 20%	18	95
5% cat instead of 20% at 90 °C	40	51
K_3PO_4 instead of Cs_2CO_3	23	95
20% Cs_2CO_3 instead of 40%	31	65
1 equiv. of $^{13}CO_2$ instead of 8 equiv.	40	65

impact of catalyst	^{13}C inc. (%)	y (%)
benzaldehyde	62	74
4-CF_3-benzaldehyde	38	66
pyridoxal phosphate	8	74
cyclohexane carboxaldehyde	10	90
acetone	14	94
acetophenone	14	97
4-Cl acetophenone	12	96

Fig. 3–7. Overview of impact of reaction parameters and catalyst structure on the carboxylate exchange of α-amino acids.

4-Anisaldehyde is the optimal catalyst for carboxylate exchange because of its balanced electrophilicity. The use of more electrophilic aryl aldehyde catalysts resulted in lower yields due to competitive trapping, while less electrophilic catalysts mediate slow rates of isotope exchange (**Fig. 3–7)**. Pyridoxal phosphate, alkyl aldehydes, and various ketones catalyze carboxylate exchange but only with low levels of incorporation (8–14%).

The impact of catalyst electrophilicity on reaction rates and α-amino acid recovery was examined in more detail (**Fig. 3–8a**). (Note that reaction sampling decreases the final ^{13}C incorporation compared to standard conditions in a sealed vial). 4-Anisaldehyde provided relatively slow rates of exchange but exhibited a long lifetime, ultimately resulting in a larger amount of labeled product. The more electrophilic catalysts 4-CF_3- or 4-CN-benzaldehyde gave fast initial rates of carboxylate exchange but are consumed quickly in the reaction (<20% catalyst remains after 1 h, compared to 60% for 4-anisaldehyde). These more electrophilic catalysts lead to the irreversible formation of the trapped aldehyde product which consume the aldehyde catalyst quickly. As a result of that, this catalyst decomposition results in worse terminal ^{13}C incorporation and reduced phenylalanine mass balance (~30% incorporation and 80% yield).

Our collaborators at Sanofi, Volker Derdau and Armin Bauer, showed that the process can be modified readily to introduce ^{14}C-labels for applications in radiolabeling studies. [^{14}C]CO_2 is generated in situ from Ba[^{14}C]CO_3 and can be used to prepare (±)-[C_1-^{14}C]phenylalanine with 53% ^{14}C incorporation, which was obtained in 51% yield after HPLC purification (11% overall radiochemical yield from Ba[^{14}C]CO_3) (**Fig. 3–8b**).

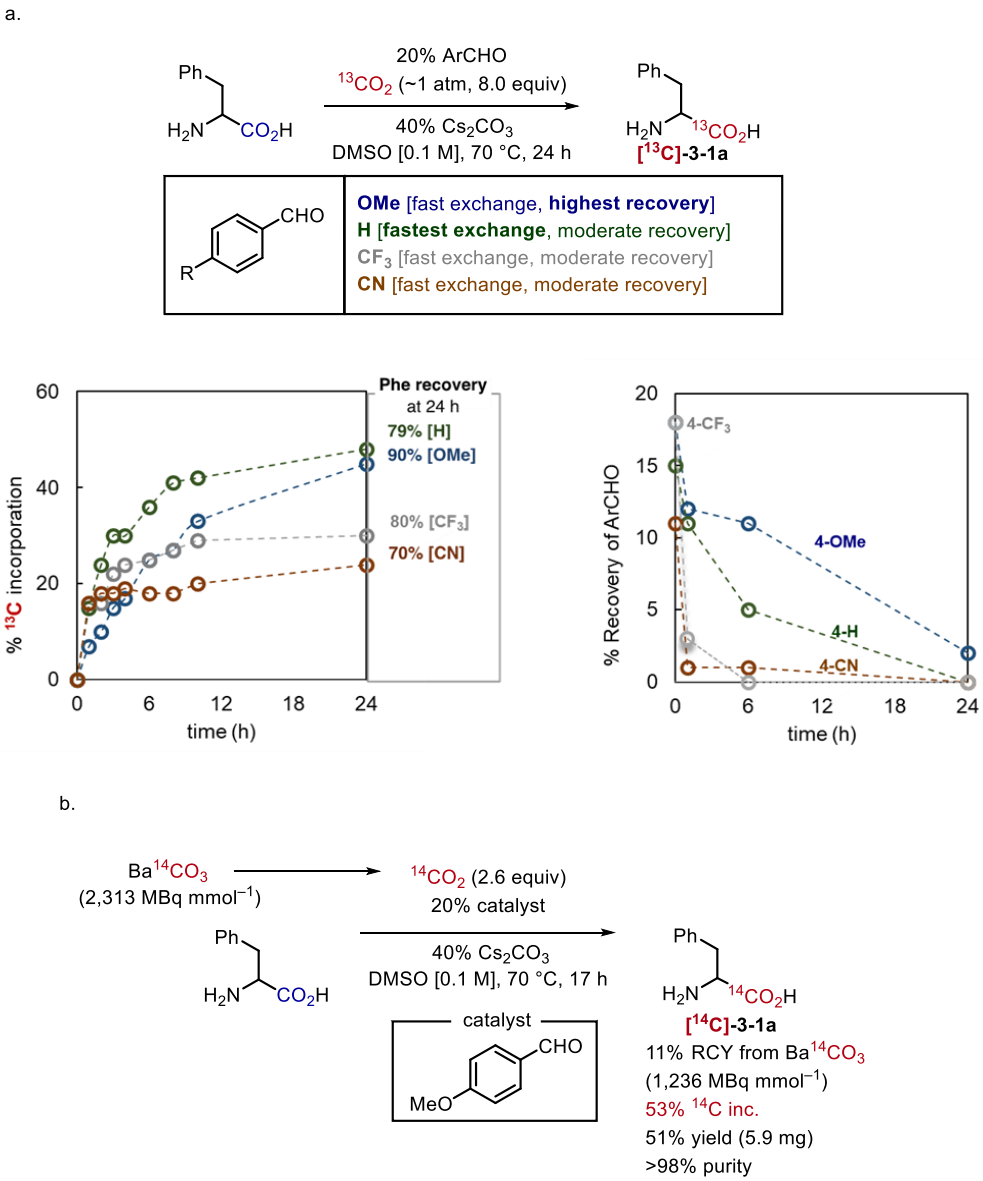


Fig. 3–8. a. Kinetic analysis and lifetime of aldehyde catalysts on the carboxylate exchange of phenylalanine, b. translation of α-amino acid carboxylate exchange to ^{14}C radiolabeling.

3.2.2 Scope of the Methodology

Aldehyde catalyzed carboxylate exchange is amenable to labeling a diverse range of unprotected α-amino acids, including most proteinogenic substrates (**Fig. 3–9a**). Aliphatic and aromatic α-amino acids can be labelled in 31–75% ^{13}C-incorporation with >50% yield (±)-phenylalanine, (±)-leucine, (**3–1**, **3–2**). In most cases, products were isolated as the corresponding *N*-tert-butyloxycarbonyl products for convenience. β-Branched aliphatic and cyclic substrates, such as (±)-valine (**3-3**, 19%) were labeled

to a lower extent under modified conditions. Acidic or basic side-chain groups generally were well-tolerated to give 23–55% incorporation of label (**3-4**, **3-5**, **3-6**, **3-7**, **3-8**, **3-9**). To gauge the potential application of the methodology for carbon-14 preclinical and clinical ADME studies, the observed [^{13}C]CO_2 incorporations can be converted to the theoretical specific activities that would result from using [^{14}C]CO_2. This is done by translation of ^{13}C incorporation to ^{14}C theoretical specific activity as shown in **Fig. 3–9b**. In general, higher incorporations of ^{14}C (≥20 μCi/mg) are required for all preclinical and clinical ADME studies.[113] Nonetheless, levels of isotope incorporation useful for ADME studies[113] were obtained in all cases, except histidine, cysteine, and threonine (**3-10**, **3-11**, **3-12**).

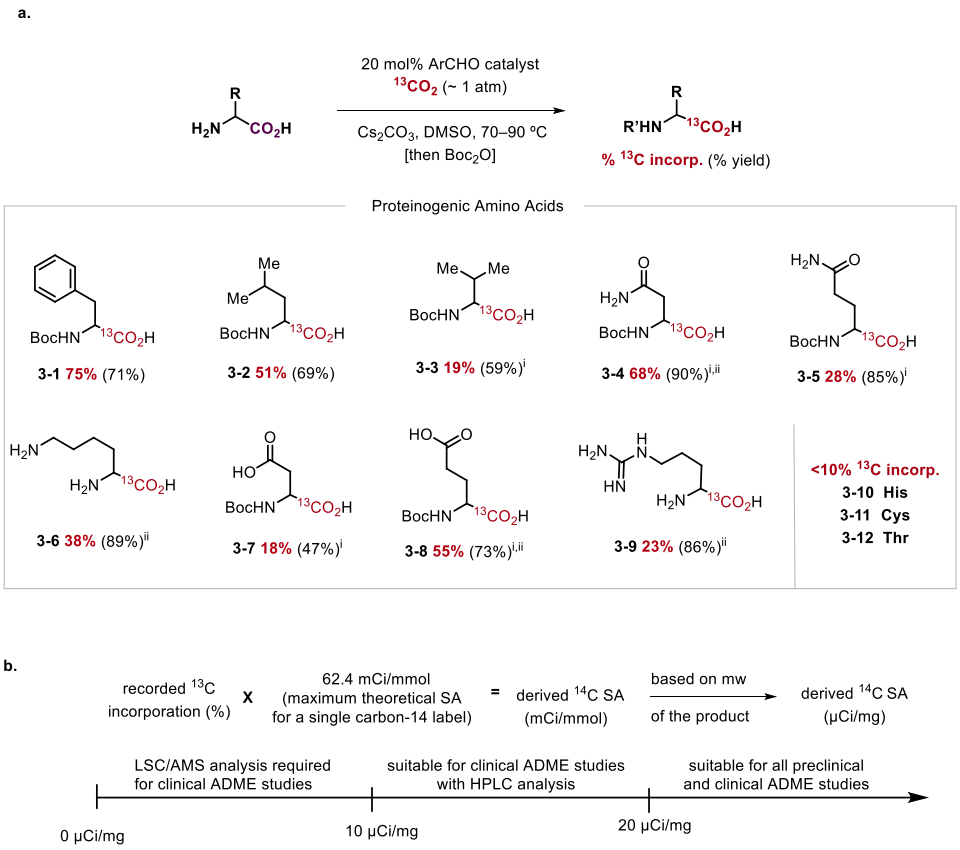


Fig. 3–9. a. Aldehyde-catalyzed carboxylate exchange of proteinogenic α-amino acids with [^{13}C]CO_2. [i] Yield determined by ^{1}H NMR spectroscopy, [ii] 75 mol% catalyst, 100% Cs_2CO_3, 0.017 M, b. Translation of ^{13}C incorporation to ^{14}C specific activity.

High label incorporation can be obtained through re-subjecting the product to the exchange conditions, for example, in the case of (**3-1a**), 96% ^{13}C labelling and 54% yield is achieved after a second exchange cycle.

Other co-workers in our lab (Mike Doyle, Chris Cooze, and Duanyang Kong) assisted with expanding the scope of the reaction. Mike helped to examine the scope of proteinogenic α-amino acids, and he did most of the non-natural α-amino acids under the standard reaction conditions shown in (**Fig. 3–10**). Chris and Duanyang conducted selected scope examples, such as (±)-glycine and (±)-serine (**3-15**, **3-16**). Mike was able to isolate other proteinogenic substrates (**Fig. 3–10a**), including aliphatic and aromatic α-amino acids, which can be labeled in 31–75% ^{13}C-incorporation with >50% yield ((±)-alanine, -isoleucine, -tyrosine, -tryptophan, -methionine (**3-13**, **3-14**, **3-17**, **3-18**). In most cases, products were isolated as the corresponding *N-tert*-butyloxycarbonyl products for convenience. Cyclic substrates, such as (±)-proline (**3-20**, 9%), were labeled to a lower extent under modified conditions. Chris showed that (±)-serine (**3-16**) could be obtained, but to a lower degree (18% and 27%, respectively).

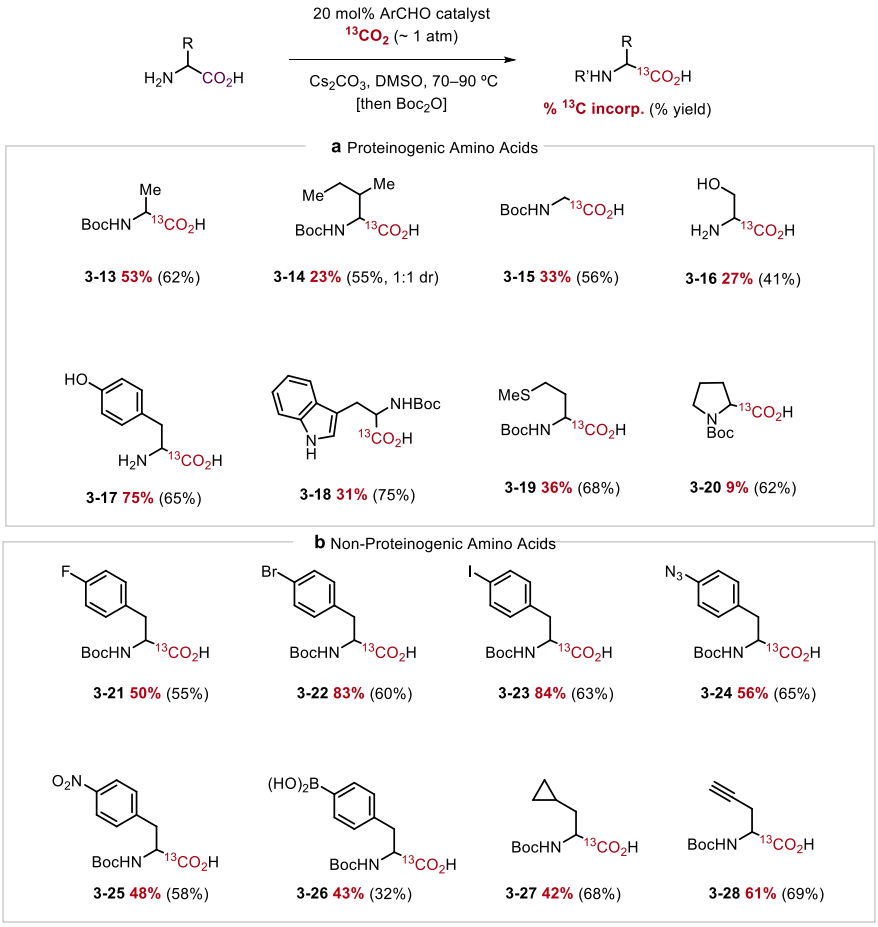


Fig. 3–10 a. Expansion of aldehyde-catalyzed carboxylate exchange of proteinogenic α-amino acids with [^{13}C]CO_2, b. Scope with non-proteinogenic α-amino acids.

It was shown that the exchange process is not impeded by various functional groups, as seen in phenylalanine derivatives containing fluoride, bromide, and iodide groups (**3-21**, **3-22**, **3-23**), or azide (**3-24**), nitro (**3-25**), and boronic acid groups (**3-26**) (**Fig. 3–10b**). Cyclopropyl (**3-27**) and terminal alkyne groups (**3-28**) smoothly undergo isotopic labeling under the standard conditions.

3.2.3 Carboxylate Exchange/Deracemization Strategies to Access Isotopically-labeled Enantiomerically Pure α-Amino Acids

Racemization of the α-amino acid occurs under the standard reaction conditions, as expected for reactions proceeding by imino carboxylate condensation intermediates.[167-168] Enantioenriched-labeled products (≥94% *ee*) can be obtained readily by established approaches. Enzymatic approach mediated kinetic resolution (KR) can be used after labeling. The first approach we followed was by using chymotrypsin[169] catalyzed ester hydrolysis, the first step for this approach, we carried out the standard reaction conditions on preformed imine carboxylate salt to get 82% NMR yield and 86% ^{13}C of the corresponding racemic labeled phenylalanine, this was followed by esterification to get the isotopically labeled phenylalanine methyl ester in 70% NMR yield, finally, chymotrypsin[169] catalyzed ester hydrolysis allowed labeled L-phenylalanine to be obtained in 99% *ee* (**3-1a** 86% ^{13}C incorp., 26% overall yield) **(Fig. 3–11a)**. The second approach we followed is by using acylase[170] catalyzed hydrolysis of *N*-acetyl leucine was used to prepare L-leucine in >99% *ee* (**3-3a** 38% ^{13}C incorp., 38% overall yield) **(Fig. 3–11b)**.

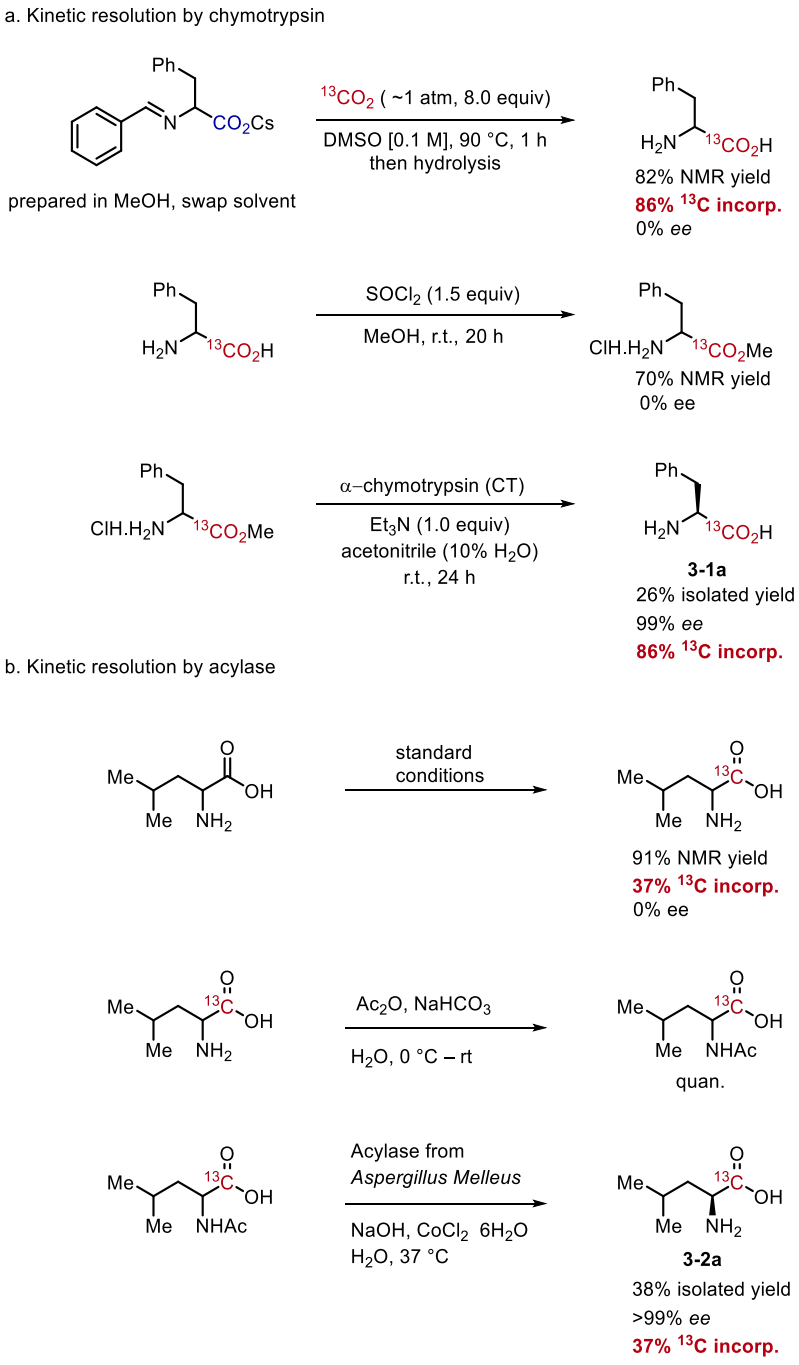


Fig. 3–11. Carboxylate exchange/deracemization strategy by enzymatic approach.

In cases where it is preferable to achieve >50% product yields, Ni-mediated dynamic kinetic resolution (DKR) can be used.[171] For example, isotopically labeled L-phenylalanine (**3-1a)** can be obtained in 63% ^{13}C incorp., 70% overall yield, 97% *ee*

by carrying out the standard reactions conditions on L-phenylalanine followed by Ni-mediated dynamic kinetic resolution (DKR) as shown in **Fig. 3–12**.

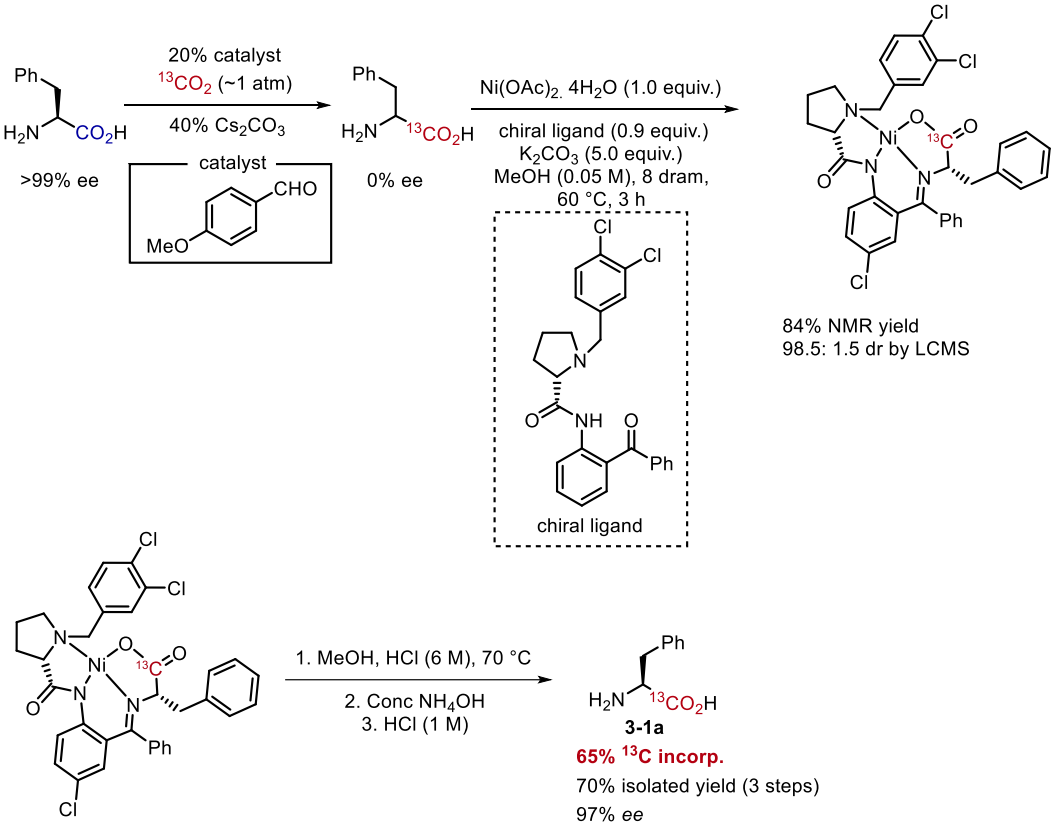


Fig. 3–12. Carboxylate exchange/deracemization strategy by Ni-mediated dynamic kinetic resolution (DKR).

We also showed that chromatographic resolution also can be accomplished readily from the corresponding (±)-Fmoc-Phe derivative (**3-1b**, 96% *ee*) **(Fig. 3–13)**.

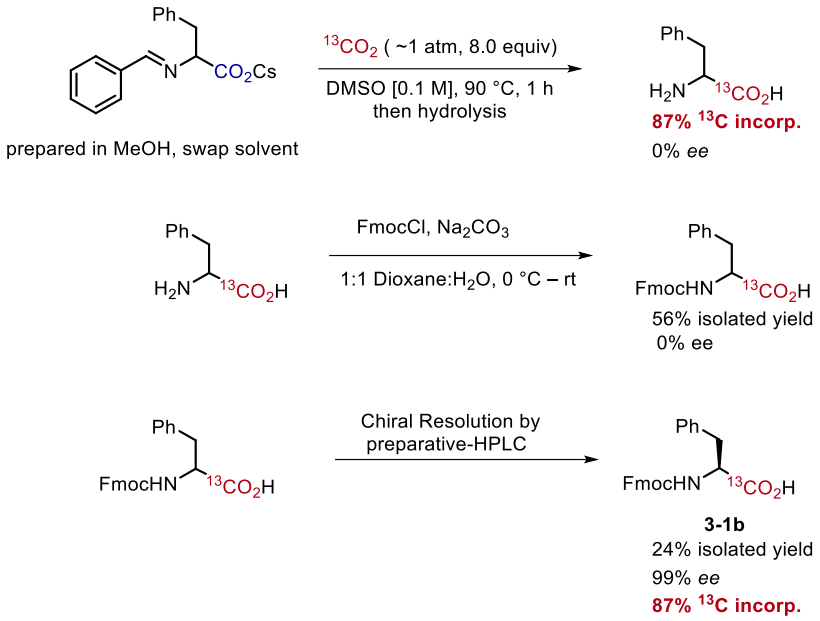


Fig. 3–13. Carboxylate exchange/deracemization strategy by preparative-HPLC.

3.2.4 Development of Fast Exchange Conditions to Enable Direct Radiolabeling of α-Amino Acids Using [^{11}C]CO_2

Modification of the reaction conditions enables direct radiolabeling of α-amino acids, using [^{11}C]CO_2. To promote faster reactions, pre-formed imine carboxylates were generated quantitatively by condensation of α-amino acids with aryl aldehydes in basic MeOH[172] and examined as reagents for carboxylate exchange. Benzaldehyde-derived imines outperformed 4-anisaldehyde or 4-cyanobenzaldehyde analogs to give 33% ^{13}C incorporation after 30 min when using 3 equivalents of [^{13}C]CO_2 , which could be improved to 62% incorporation at 90 ºC (**Fig. 3–14a**). Our collaborators at Ottawa, Maxime Munch and Braeden Mair, showed that these conditions can be modified readily to introduce ^{11}C-labels for applications in radiolabeling studies. Translation of these conditions allowed for the preparation of (±))-[C_1-^{11}C]phenylalanine in 24% radiochemical yield (RCY) (**Fig. 3–14b**).

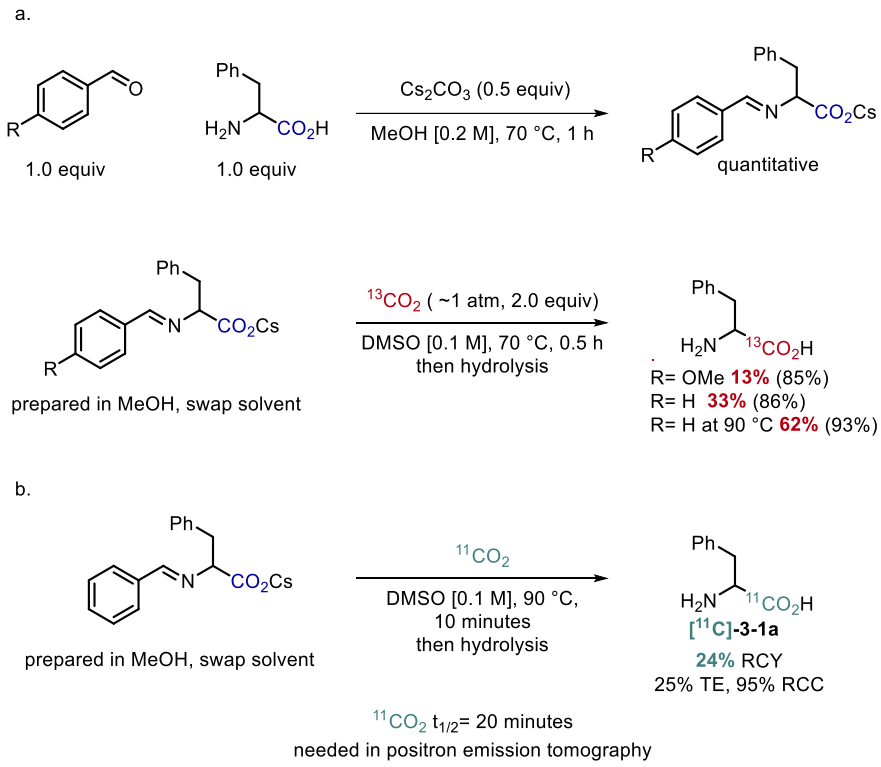


Fig. 3–14 a. Development of fast conditions for carboxylate exchange. b. Translation of reaction to radiolabeling with [^{11}C]CO_2.

3.2.5 Mechanistic Studies

The mechanism for carboxylate exchange likely occurs by the carboxylation of imino-enol or imino dienolate intermediates[173] followed by decarboxylation[174] (**Fig. 3–15a**, **i-1** to [C_1-^{13}C]-**3-1aa**, rather than by trapping from an aza-allyl anion formed by an initial decarboxylation (**Fig. 3–15a**, **i-3**).[175]

A key mechanistic observation from reactions conducted by co-workers in the group showed that when D_2O is added to a reaction conducted under otherwise standard conditions, using [^{12}C]-**3-1**, products arising from both H/D and ^{12}C/^{13}C exchange are observed (**Fig. 3–15a**).[176] The presence of D_2O slows the rate of carboxylate exchange; however, the amount of proto- or deuterodecarboxylation product remains low (<1%). Assuming that the exchange processes occur from a common intermediate, these results suggest that an aza-allyl anion is not an intermediate formed during the reaction. It also was shown that any amine generated from α-amino acid protodecarboxylation is unable to re-enter the exchange pathway via carboxylation (**Fig. 3–15b**).[175,177]

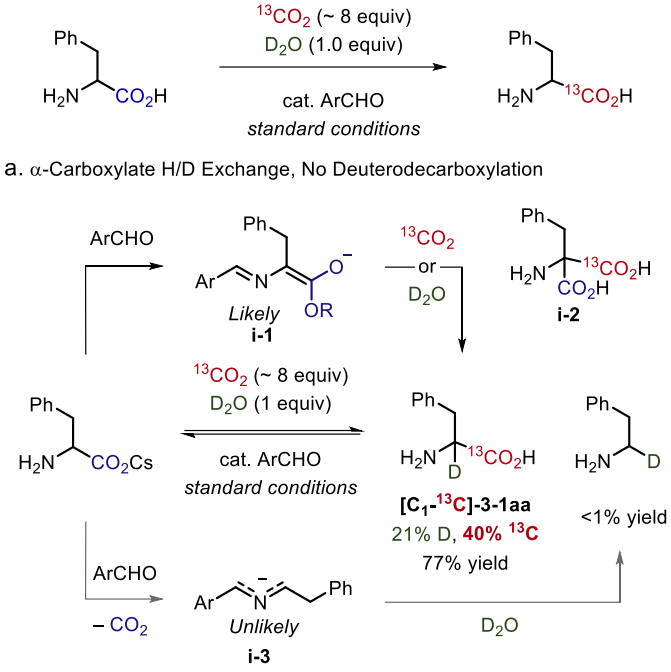


Fig. 3–15. Mechanistic experiments. a. α-carboxylate H/D exchange. b. Attempt to carboxylate the imine.

To understand the nature of intermediates better, whether it is an imino-enol, imino dienolate intermediates, or aza-allyl anion, we looked at the reactivity of enolizable α-amino acid versus non-enolizable non natural α-amino acid under our carboxylate exchange conditions . We observed that acetone catalyzes the carboxylate exchange of phenylalanine via reactive imine (**i-4**), leading to 14% exchange in [C_1-^{13}C]-**3-1a**. On the other hand, non-enolizable α-amino acid 2-aminoisobutyric acid (**3-29**) was unreactive to carboxylate exchange, despite the ability of this substrate (**3-29**) to form a benzylic stabilized aza-allyl anion by decarboxylation of imine **i-5** (**Fig. 3–**

16). These results suggest that an aza-allyl anion is not an intermediate formed during the reaction.

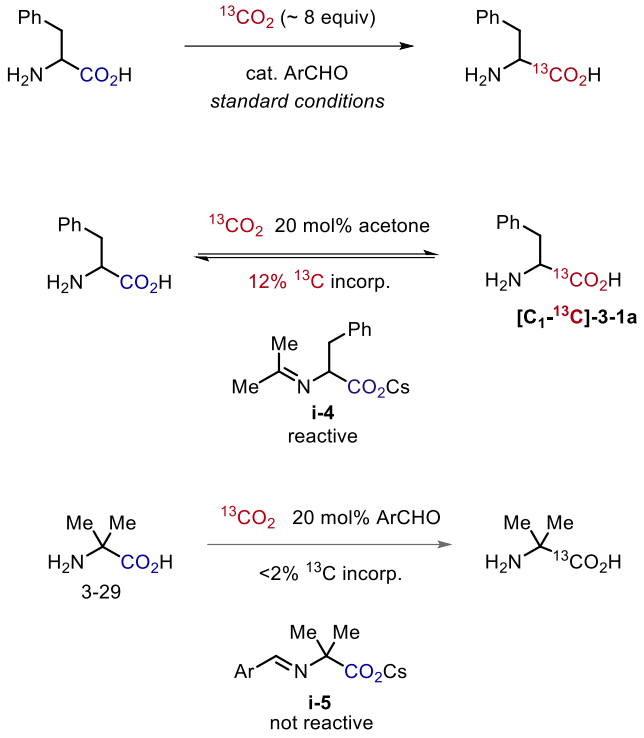


Fig. 3–16. Mechanistic experiments, the reactivity of enolizable α-amino acid versus non-enolizable α-amino acid under standard reaction conditions.

The positional selectivity of carboxylation from pseudo-symmetrical intermediates generated by condensation between 4-bromophenylalanine and phenylacetaldehyde also supports the formation of a malonate intermediate **i-6,** rather than an aza-allyl intermediate **i-7** that would generate some amount of phenylalanine (**Fig. 3–17**). In this experiment we did not detect any amount of phenylalanine by LC-MS or NMR which suggests that an aza-allyl intermediate is not form during the cycle. In addition to that, when conducting carboxylate exchange reactions, the corresponding arylglycine regioisomers were not observed at various aldehyde loadings, further suggesting that an aza-allyl nucleophile like **i-3** is not generated.

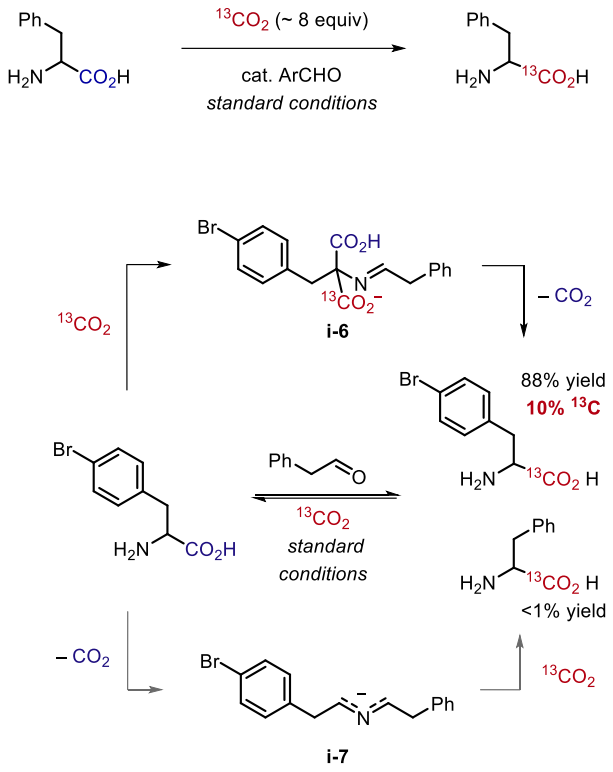


Fig. 3–17. Mechanistic experiments, positional selectivity of carboxylation.

A potential mechanism for the aryl aldehyde catalyzed carboxylate exchange is provided in (**Fig. 3–18**). Condensations of aryl aldehyde (**3-A**) and α-amino acid (**3-B**) gives an imine intermediate (**3-C**). This imine intermediate can undergo decarboxylation to give aza-allyl intermediate (**3-D**), which is unlikely as we showed in the previous mechanistic probes. The most plausible pathway for the mechanism is that a reactive imine carboxylate nucleophile (**3-E**) can be generated by either deprotonation or enol formation. This species can undergo reversible carboxylation with dissolved CO_2 to form an iminomalonate intermediate (**3-F**). Amino malonate (**3-F**) undergoes decarboxylation to give the isotopically labeled enolate imine intermediate (**3-G**), which upon protonation gives the isotopically labeled imine (**3-H**), then hydrolysis to give the isotopically labeled α-amino acid (**3-I**). It is likely that all steps in the cycle are reversible, thus, the maximum amount of *CO_2 incorporated is under thermodynamic control.

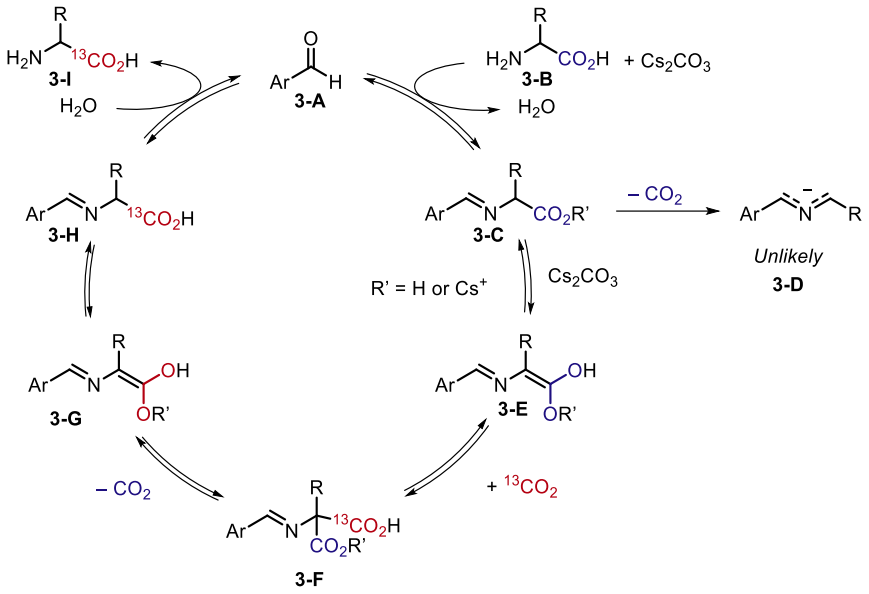


Fig. 3–18. Potential mechanism of the aldehyde-catalyzed isotopic carboxylate exchange of α-amino acids, proton transfer steps omitted for clarity.

3.3 Summary and Conclusions

In summary, we have reported a new strategy for the isotopic labeling of α-amino acids catalyzed by aryl aldehydes with conjunction of $[^{13}C]CO_2$ via a reversible decarboxylation/carboxylation event of imine carboxylate intermediate. Productive catalysts must have balanced electrophilicity to enable carboxylate exchange without being consumed irreversibly in the process. 4-Anisaldehyde was the best catalyst in terms of the amount of incorporation and yield of the product we obtained. The more electrophilic catalysts 4-CF_3- or 4-CN-benzaldehyde gave fast initial rates of carboxylate exchange but are consumed quickly in the process due to irreversible formation of the trapped aldehyde product which consume these electrophilic catalysts quickly. Mechanistic studies indicate that the reaction likely proceeds via the trapping of $*CO_2$ by imine-carboxylate intermediates to generate aminomalonates that are prone to monodecarboxylation. The identification of the intermediate has allowed for the rapid and late-stage ^{11}C-radiolabeling of α-amino acids in the presence of $[^{11}C]CO_2$ through the pre-generation of the imine carboxylate intermediate.

Our developed method granted access to C1-labeled products in a direct and operationally trivial manner. Given the widespread use of labeled α-amino acids in

discovery science, drug development, and medical imaging, we expect this finding to have immediate application.

One of the shortcomings of the process include the racemization of enantiopure α-amino acids during labeling. This drawback motivated us to develop a new method for the synthesis of enantioriched isotopically labeled α-amino acids, as will be discussed in Chapter 4.

3.4 Procedures and Characterization

General Considerations:

Unless noted, all reactions were conducted under an inert atmosphere employing standard Schlenk techniques or by the use of an N_2-filled glovebox. All glassware was oven-dried prior to use. Flash chromatography was performed, as described by Still and co-workers[128] (SiliaFlash P60, 40-63μm, 60A silica gel, Silicycle) or by automated flash chromatography (Silicycle SiliaSep premium 12g). Analytical thin-layer chromatography was performed, using glass plates pre-coated with silica (SiliaPlate G TLC - Glass-Backed, 250μm, Silicycle). TLC plates were visualized by staining with aqueous acidic ninhydrin and/or by UV light. NMR spectra (^{1}H, ^{13}C, ^{19}F, ^{77}Se) were obtained on an Agilent VNMRS 700 MHz, Varian VNMRS 600 MHz, Varian VNMRS 500 MHz, or Varian VNMRS 400 MHz spectrometer. The chemical shifts are given as parts per million (ppm) and were referenced to the residual solvent signal ($CDCl_3$: δH = 7.26 ppm, δC = 77.06 ppm) (DMSO-d_6: δH = 2.49 ppm, δC = 39.50 ppm) (Acetone-d_6: δH = 2.04 ppm, δC = 29.80 ppm) (MeOH-d_4: δH = 3.30 ppm, δC = 49.00 ppm). HRMS analyses of ^{13}C labelled compounds were performed on an Agilent Technologies 6220 oaTOF instrument (ESI, APPI, APCI) in positive or negative ionization mode. Crude LC-MS analyses of ^{13}C labelled compounds were performed on an Agilent Technologies 1260 system with G6130B MSD single quadrupole MS (ESI) in positive or negative ionization mode, using a Phenomenex Kinetex C8 column (2.1 × 50 mm, 1.7 μm particle size) or a Phenomenex Luna Omega C18 Polar column (2.1 × 50 mm, 1.6 μm particle size) in reverse-phase. Chiral HPLC analysis was accomplished on an Agilent Technologies 1260 system with Daicel ChiralPak IA, IC, or IG columns (4.6 × 150 mm, 5 μm particle size) under normal-phase conditions or an

Agilent Technologies 1100 system with an Astec Chirobiotic-T column (4.6 × 100 mm, 5 µm particle size) under reverse-phase conditions. Preparative HPLC was accomplished, using an Agilent 1260 Infinity system under normal-phase (Phenomenex Lux i-Amylose-3 column, 10 × 250 mm, 5 µm particle size) and reverse-phase conditions (Agilent Prep-C18 column, 21.2 × 150 mm, 10 µm particle size). Unless otherwise noted, quantitative ^{1}H NMR yields were determined from crude reaction mixtures, using 3-(trimethylsilyl)-1-propanesulfonic acid (DSS) as an internal standard. DSS was found to be stable under the reaction conditions by determining its concentration by the addition of a second internal standard (trimethoxybenzene) that was added after the reaction was quenched. Unless otherwise noted, all reagents were obtained from commercial vendors (Sigma-Aldrich, Combi-Blocks, Alfa Aesar, Acros, and TCI) and used as supplied. [^{13}C]CO_2 (99.0 atom % ^{13}C) and anhydrous DMSO were purchased from Sigma-Aldrich. Chiral ligand for the preparation of **3-1a** was synthesized according to the literature procedure.[171] The preparation of the pre-formed imines from the corresponding amino acids and aldehydes (General Procedure E) was adapted from a literature procedure.[172]

General Procedures and Characterization for Section 3.2

General Procedure A (amino acids with hydrophobic groups): In an atmosphere-controlled glovebox (not required, see below), amino acid (0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.), and sodium trimethylsilylpropanesulfonate internal standard (DSS) were added sequentially to an 8-dram vial charged with a stir bar, followed by the addition of anhydrous DMSO (2.0 mL). The vial was sealed with a PTFE-lined cap and removed from the glovebox. The reaction headspace was evacuated on a Schlenk line (~300 mtorr), using a 25-gauge needle. The vial headspace was refilled carefully with 15 psi [^{13}C]CO_2 through the PTFE-lined cap with a 25-gauge needle until the internal pressure reached ~1 atm (requires 20–60 sec, depending on the pressure of the [^{13}C]CO_2 tank). This provides ~8 equivalents (~1.6 mmol) of [^{13}C]CO_2, which would result in an equilibrium exchange incorporation of ~85%. Next, the vial cap was sealed with parafilm and electrical tape, and the reaction was stirred at 70 ˚C in an aluminum block.

Upon completion of the reaction (25–48 h), the vial was cooled to room temperature. To determine the percent recovery of the amino acid, a small aliquot (~5 μL) of the reaction was placed in 0.70 mL D_2O for calibrated ^1H NMR analysis, using DSS as the reference signal. After sampling, the reaction headspace was evacuated and refilled with N_2. This cycle was repeated three times. Then, Boc_2O (91.9 μL, 0.40 mmol, 2.0 equiv.) was added via syringe, and the reaction was stirred overnight at room temperature. After overnight stirring, a small aliquot of the reaction (~5 μL) was placed in 1.0 mL of 1:1 MeOH:H_2O/0.1% HCO_2H for LC-MS analysis to determine the crude ^{13}C% incorporation of the amino acid. The reaction mixture was diluted with H_2O (10 mL) and acidified to pH 1–2, using 1M HCl (2–3 mL) and the aqueous layer was extracted with EtOAc (5 × 10 mL). Next, the combined organics were washed with H_2O (1 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo, and purified by silica gel chromatography. The ^{13}C% incorporation was obtained through high resolution mass spectrometry (HRMS) analysis of the products. Note: Boc protection of the products was done for convenience of isolation; this is not an essential step for carboxylate exchange. Note: for a 0.05 mmol scale reactions, use a 2-dram vial, and for a 0.10 mmol scale reactions, use a 4-dram vial. A similar equilibrium incorporation (~85%) would be obtained under these conditions.

General Procedure B (amino acids with polar charged groups, polar uncharged groups, and special cases): In an atmosphere-controlled glovebox, amino acid (0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (10.2 mg, 0.075 mmol, 0.75 equiv.), Cs_2CO_3 (32.5 mg, 0.10 mmol, 1.0 equiv.), and sodium trimethylsilylpropanesulfonate internal standard (DSS) were added sequentially to an 8-dram vial charged with a stir bar, followed by the addition of anhydrous DMSO (6.0 mL). The vial was sealed with a PTFE-lined cap and removed from the glovebox. The reaction headspace was evacuated on a Schlenk line (~300 mtorr), using a 25-gauge needle. The vial headspace was carefully refilled with 15 psi [^{13}C]CO_2 through the PTFE-lined cap with a 25 gauge needle until the internal pressure reached ~1 atm (requires 20–60 sec, depending on the pressure of the [^{13}C]CO_2 tank). This provides ~10 equivalents (~1.0 mmol) of

[^{13}C]CO_2, which would result in an equilibrium exchange incorporation of ~90%. The vial cap was sealed with parafilm and electrical tape, and the reactions were stirred at 70 ˚C in an aluminum block. Upon completion of the reaction (48 h), the vial was cooled to room temperature. To determine the percent recovery of the amino acid, a small aliquot (~25 µL) of the reaction was placed in 0.70 mL D_2O for calibrated ^{1}H NMR analysis, using DSS as the reference signal. After sampling, the reaction headspace was evacuated and refilled with N_2. This cycle was repeated three times. Then, Boc_2O (45.9 µL, 0.20 mmol, 2.0 equiv.) was added via syringe, and the reaction was stirred overnight at room temperature. After overnight stirring, a small aliquot of the reaction (~25 µL) was placed in 1.0 mL of 1:1 MeOH:H_2O/0.1% HCO_2H for LC-MS analysis to determine the crude ^{13}C% incorporation of the amino acid.

General Procedure C (no glovebox, amino acids with hydrophobic groups): Amino acid (0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.), and sodium trimethylsilylpropanesulfonate internal standard (DSS) were added sequentially to an 8-dram vial charged with a stir bar, followed by the addition of anhydrous DMSO (2.0 mL), and the vial was sealed with a PTFE-lined cap. The reaction headspace was evacuated on a Schlenk line (~300 mtorr), using a 25-gauge needle and subsequently backfilled with N_2. This cycle was repeated three times. The reaction headspace was evacuated again (~300 mtorr) and then refilled carefully with 15 psi [^{13}C]CO_2 through the PTFE-lined cap, using a 25-gauge needle until the internal pressure reached ~1 atm (requires 20–60 sec, depending on the pressure of the [^{13}C]CO_2 tank). This provides ~8 equivalents (~1.6 mmol) of [^{13}C]CO_2, which would result in an equilibrium exchange incorporation of ~85%. Then, the vial cap was sealed with parafilm and electrical tape, and the reaction was stirred at 70 ˚C in an aluminum block. Upon completion of the reaction (25–48 h), the vial was allowed to cool to room temperature. The remainder of the procedure is identical to the general procedure A.

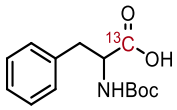
General Procedure D (no glovebox, amino acids with polar charged groups, polar uncharged groups, and special cases): Amino acid (0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (10.2 mg, 0.075 mmol, 0.75 equiv.), Cs_2CO_3 (32.5 mg, 0.10

mmol, 1.0 equiv.), and sodium trimethylsilylpropanesulfonate internal standard (DSS) were added sequentiallyt to an 8-dram vial charged with a stir bar, followed by the addition of anhydrous DMSO (6.0 mL), and the vial was sealed with a PTFE-lined cap. The reaction headspace was evacuated on a Schlenk line (~300 mtorr), using a 25-gauge needle and subsequently backfilled with N_2. This cycle was repeated three times. The reaction headspace was evacuated again (~300 mtorr) and then refilled carefully with 15 psi [^{13}C]CO_2 through the PTFE-lined cap, using a 25-gauge needle until the internal pressure reached ~1 atm (requires 20–60 sec, depending on the pressure of the [^{13}C]CO_2 tank). This provides ~10 equivalents of (~1.0 mmol) [^{13}C]CO_2, which would result in an equilibrium exchange incorporation of ~90%. Then, the vial cap was sealed with parafilm and electrical tape, and the reaction was stirred at 70 ˚C in an aluminum block. Upon completion of the reaction (48 h), the vial was allowed to cool to room temperature. The remainder of the procedure is identical to the general procedure B.

General Procedure E (standard reaction, using the pre-formed imine from the corresponding amino acid and aldehyde): In an atmosphere-controlled glovebox, amino acid (0.10 mmol, 1.0 equiv.), benzaldehyde (10.6 mg, 0.10 mmol, 1.0 equiv.), Cs_2CO_3 (16.3 mg, 0.05 mmol, 0.50 equiv.), 1,3,5-trimethoxybenzene internal standard (TMB), and anhydrous MeOH (0.5 mL) were added sequentially to a dried 1-dram vial charged with a stir bar. The vial was sealed with a PTFE-lined cap and removed from the glovebox. The reaction was stirred at 70 °C for 1 h in an aluminum block, and then the reaction was cooled to room temperature. The solvent was evaporated in vacuo to isolate the imine salt (mixed with TMB). Then, the vial was evacuated and refilled with N_2 on a Schlenk line. This cycle was repeated three times. After the purging cycle, the vial was taken back into the glovebox, and sodium trimethylsilylpropanesulfonate internal standard (DSS) was added, followed by the addition of anhydrous DMSO (1.0 mL). The vial was sealed with a PTFE-lined cap and removed from the glovebox. The reaction headspace was evacuated (~300 mtorr), using a 25-gauge needle. Then, the vial headspace was refilled carefully with 15 psi [^{13}C]CO_2 through the PTFE-lined cap, using a 25-gauge needle until the internal pressure reached ~1 atm (requires 20–60 sec, depending on the pressure of the [^{13}C]CO_2 tank). This provides ~8 equivalents of (~0.8

mmol) [^{13}C]CO_2, which would result in an equilibrium exchange incorporation of ~85%. Then, the vial cap was sealed with parafilm and electrical tape, and the reaction was stirred at 90 ˚C in an aluminum block. Upon completion of the reaction (1 h), the vial was cooled to room temperature. The remainder of the procedure is identical to the general procedure A.

Specific Experimental Details and Product Characterization Data



3-1 Prepared according to the general procedure A from ^{12}C-phenylalanine (33.0 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 84%. Isolated in 71% yield, 75% ^{13}C incorporation (HRMS) as an off-white solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap. Through 17 independent experiments conducted over the course of the project, the average crude ^{1}H NMR yield and % ^{13}C incorporation was 84% and 71%, respectively.

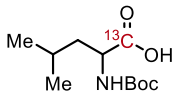
3-1 also can be labeled with >95% ^{13}C incorporation by performing a recycling experiment, in which the substrate is subjected to the reaction conditions twice. Prepared according to the General Procedure A from ^{12}C-phenylalanine (16.5 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (2.7 mg, 0.02 mmol, 0.20 equiv.), and Cs_2CO_3 (13.0 mg, 0.04 mmol, 0.40 equiv.) in DMSO (1.0 mL). The reaction mixture was allowed to stir for 28 h. Then, the crude reaction mixture was lyophilized to remove the DMSO, and the vial was evacuated and refilled with N_2; this cycle was repeated thee times. After the purging cycle, the vial was taken back into the glovebox, and the product was prepared according to the general procedure A, using 4-methoxybenzaldehyde (2.7 mg, 0.02 mmol, 0.20 equiv.), Cs_2CO_3 (13.0 mg, 0.04 mmol, 0.40 equiv.), and DMSO (1.0 mL). The reaction was allowed to stir for 28 h.

The reaction was not subjected to Boc protection and was analyzed directly by HRMS. ^{1}H NMR yield: 54%, 96% ^{13}C incorporation (HRMS).

^{1}H NMR ($CDCl_3$, 500 MHz) (~3:1 mixture of rotamers) δ 7.36–7.18 (m, 5H), 6.07 (br s, 0.3H), 4.98 (br s, 0.7H), 4.64 (br s, 0.7H), 4.43 (br s, 0.3H), 3.23 (dd, *J* = 13.8, 5.4 Hz, 1H), 3.11 (dd, *J* = 13.8, 8.8 Hz, 0.7H), 2.95 (br s, 0.3 H), 1.45 (s, 6.7H), 1.36 (s, 2.3H);

^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 176.5 (176.4), 155.4 (156.1), 135.9 (136.2), 129.4, 128.6, 127.1, 80.3 (81.5), 54.3 (d, *J* = 60.6 Hz) (56.0), 37.8 (39.0), 28.3 (29.7);

HRMS (ESI): calcd. for $C_{13}[^{13}C]H_{18}NO_4$ $[M-H]^-$: 265.1275. Found 265.1277.

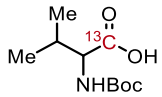


3-2 Prepared according to the general procedure A from ^{12}C-leucine (26.2 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 82%. Then, the reaction mixture was diluted with H_2O (10 mL) and acidified to pH 1–2, using 1M HCl (2–3 mL). The aqueous layer was extracted with EtOAc (5 × 10 mL), and the combined organics were washed with H_2O (1 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Isolated in 69% yield, 51% ^{13}C incorporation (HRMS) as a white solid after the workup step. No silica gel chromatography was necessary for purification.

^{1}H NMR ($CDCl_3$, 500 MHz) (~3:1 mixture of rotamers) δ 5.51 (br s, 0.2H), 4.88 (br d, *J* = 8.2 Hz, 0.8H), 4.34 (br s, 0.8H), 4.18 (br s, 0.2H), 1.83–1.62 (m, 2H), 1.62–1.52 (m, 1H), 1.47 (s, 8.5H), 1.28 (s, 0.5H), 0.99 (d, *J* = 6.5 Hz, 6H);

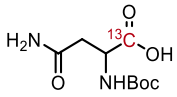
^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.8, 155.7, 80.3, 52.0 (d, *J* = 57.7 Hz), 28.3, 24.8, 22.9, 21.8;

HRMS (ESI): calcd. for $C_{10}[^{13}C]H_{20}NO_4$ $[M-H]^-$: 231.1431. Found 231.1431.



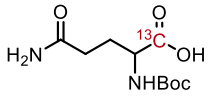
3-3 The experiment followed the general procedure A from ^{12}C-valine (23.4 mg, 0.20 mmol, 1.0 equiv.), benzaldehyde (15.9 mg, 0.15 mmol, 0.75 equiv.), and Cs_2CO_3 (65.2 mg, 0.20 mmol, 1.0 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 48 h at 80 ˚C. ^{1}H NMR yield: 50%. ^{13}C incorporation (LCMS): 18%.

LRMS (ESI): calcd. for $C_9[^{13}C]H_{18}NO_4$ $[M-H]^-$: 217.13. Found 217.09.



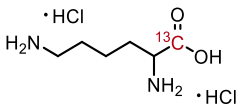
3-4 The experiment followed the general procedure B from ^{12}C-asparagine (13.2 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (10.2 mg, 0.075 mmol, 0.75 equiv.), and Cs_2CO_3 (32.5 mg, 0.10 mmol, 1.0 equiv.) in DMSO (6.0 mL). The reaction mixture was allowed to stir for 48 h. ^{1}H NMR yield: 90%. ^{13}C incorporation (LCMS): 68%.

LRMS (ESI): calcd. for $C_8[^{13}C]H_{15}N_2O_5$ $[M-H]^-$: 232.10. Found 232.07.



3-5 The experiment followed the general procedure B from ^{12}C-glutamine (14.6 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (10.2 mg, 0.075 mmol, 0.75 equiv.), and Cs_2CO_3 (32.5 mg, 0.10 mmol, 1.0 equiv.) in DMSO (1.0 mL). The reaction mixture was allowed to stir for 48 h. ^{1}H NMR yield: 85%. ^{13}C incorporation (LCMS): 28%.

LRMS (ESI): calcd. for $C_9[^{13}C]H_{17}N_2O_5$ $[M-H]^-$: 246.12. Found 246.10.



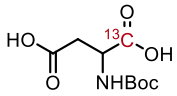
3-6 (±)-[^{13}C]-lysine dihydrochloride prepared according to the general procedure B from L-lysine (14.6 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde

(10.2 mg, 0.075 mmol, 0.75 equiv.), and Cs_2CO_3 (32.5 mg, 0.10 mmol, 1.0 equiv.) in DMSO (6.0 mL). The reaction mixture was allowed to stir for 48 h. ^{1}H NMR yield: >95%. The crude reaction mixture was not subjected to Boc_2O protection. The crude mixture was lyophilized to get rid of the DMSO. This was followed by dissolving the crude powder in H_2O (10 mL), then acidifying to pH 1–2 ,using 1M HCl (2–3 mL). The aqueous solution was washed with EtOAc (3 × 10 mL) to remove the aldehyde, and the aqueous layer was lyophilized again to afford a yellow solid. Isolated in 89% yield (19.6 mg), 38% ^{13}C incorporation (HRMS) as a white solid after purification by preparative-HPLC (Phenomenex Synergi 5u Hydro-RP 80Å 150 mm × 10 mm, 4mm, 0% MeCN in H_2O to 5% MeCN in H_2O).

^{1}H NMR (D_2O, 500 MHz) δ 4.08–4.03 (m, 1H), 3.02 (t, *J* = 7.8 Hz, 2H), 2.06–1.90 (m, 2H), 1.77–1.68 (m, 2H), 1.61–1.42 (m, 2H);

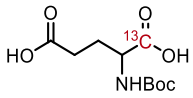
^{13}C NMR (D_2O, 125 MHz) δ 172.3, 52.9 (d, *J* = 58.9 Hz), 38.8, 29.4, 26.3, 21.5;

HRMS (ESI): calcd. for $C_5[^{13}C]H_{15}N_2O_2$ $[M+H]^+$: 148.1162. Found 148.1159.



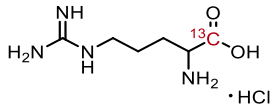
3-7 The experiment followed the general procedure B from ^{12}C-aspartic acid (13.3 mg, 0.10 mmol, 1.0 equiv.), benzaldehyde (8.0 mg, 0.075 mmol, 0.75 equiv.), and Cs_2CO_3 (32.5 mg, 0.10 mmol, 1.0 equiv.) in DMSO (1.0 mL). The reaction mixture was allowed to stir for 48 h. ^{1}H NMR yield: 47%. ^{13}C incorporation (LCMS): 18%.

LRMS (ESI): calcd. for $C_8[^{13}C]H_{14}NO_6$ $[M-H]^-$: 233.09. Found 233.00.



3-8 The experiment followed the general procedure B from ^{12}C-glutamic acid (14.7 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (10.2 mg, 0.075 mmol, 0.75 equiv.), and Cs_2CO_3 (32.5 mg, 0.10 mmol, 1.0 equiv.) in DMSO (6.0 mL). The reaction mixture was allowed to stir for 48 hs. ^{1}H NMR yield: 73%. ^{13}C incorporation (LCMS): 55%.

LRMS (ESI): calcd. for $C_9[^{13}C]H_{18}NO_4S$ $[M-H]^-$: 247.10. Found 247.00.



3-9 (±)-[^{13}C]-arginine hydrochloride prepared according to the general procedure B from L-arginine (17.4 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (10.2 mg, 0.075 mmol, 0.75 equiv.), and Cs_2CO_3 (32.5 mg, 0.10 mmol, 1.0 equiv.) in DMSO (6.0 mL). The reaction mixture was allowed to stir for 48 h. ^{1}H NMR yield: 85%. The crude reaction mixture was not subjected to Boc_2O protection. The crude mixture was lyophilized to get rid of the DMSO, and this was followed by dissolving the crude powder in H_2O (10 mL), then acidifying to pH 1–2, using 1M HCl (2–3 mL). The aqueous solution was washed with EtOAc (3 × 10 mL) to remove the aldehyde, and the aqueous layer was lyophilized again to afford a yellow solid. Isolated in 84% yield (17.8 mg), 23% ^{13}C incorporation (HRMS) as a white solid after purification by preparative-HPLC (Phenomenex Synergi 5u Hydro-RP 80Å 150 mm × 10 mm, 4mm, 0% MeCN in H_2O to 5% MeCN in H_2O).

^{1}H NMR (D_2O, 500 MHz) 4.10 (app t, J = 6.5 Hz, 1H), 3.24 (t, J = 6.5 Hz, 2H), 2.0–1.91 (m, 2H), 1.82–1.63 (m, 2H);

^{13}C NMR (D_2O, 125 MHz) δ 172.0, 156.8, 52.7, 40.4, 27.0, 23.8;

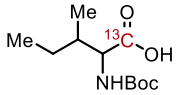
HRMS (ESI): calcd. for $C_5[^{13}C]H_{15}N_4O_2$ $[M+H]^+$: 176.1223. Found 176.1221.

3-13 Prepared according to the general procedure A from ^{12}C-alanine (17.8 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 72%. Isolated in 62% yield, 53% ^{13}C incorporation (HRMS) as an off-white solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~5:2 mixture of rotamers) δ 6.55 (br s, 0.3H), 5.07 (br s, 0.7H), 4.37 (br s, 0.7H), 4.20 (br s, 0.3H), 1.50–1.44 (m, 12H);

^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 177.9 (177.5), 155.5 (156.5), 80.3 (81.5), 49.1 (d, J = 58.2 Hz) (49.4), 28.3, 18.3;

HRMS (ESI): calcd. for $C_7[^{13}C]H_{14}NO_4$ $[M-H]^-$: 189.0962. Found 189.0963.



3-14 Prepared according to the general procedure A from ^{12}C-isoleucine (26.2 mg, 0.20 mmol, 1.0 equiv.), benzaldehyde (15.9 mg, 0.15 mmol, 0.75 equiv.), and Cs_2CO_3 (65.2 mg, 0.20 mmol, 1.0 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 48 h at 80 ˚C. ^{1}H NMR yield: 73%. Isolated in 55% yield, 23% ^{13}C incorporation (HRMS), 50:50 *dr* as an off-white solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~1:1 mixture of diastereomers, ~2.5:1 mixture of rotamers) δ 5.69 (br s, 0.2H), 5.39 (m, 0.2H), 5.13–4.97 (m, 0.8H), 4.47–4.24 (m, 0.8H), 2.06–1.90 (m, 1H), 1.48 (s, 8H), 1.40–1.20 (m, 3H), 1.03–0.86 (m, 6H);

^{13}C NMR ($CDCl_3$, 125 MHz) (diastereomer) δ 177.5 (177.0), 155.9 (155.7), 80.9, 57.8 (56.6), 37.7 (37.4), 28.3, 26.3 (24.9), 15.6 (14.4), 11.7 (11.6);

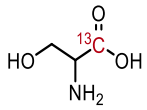
HRMS (ESI): calcd. for $C_{10}[^{13}C]H_{20}NO_4$ $[M-H]^-$: 231.1431. Found 231.1430.

3-15 Prepared according to the general procedure A from ^{12}C-glycine (15.0 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (20.4 mg, 0.15 mmol, 0.75 equiv.), and Cs_2CO_3 (65.2 mg, 0.20 mmol, 1.0 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 48 h. ^{1}H NMR yield: 84%. Isolated in 56% yield, 33% ^{13}C incorporation (HRMS) as an off-white oil after purification by silica gel chromatography (3% to 7% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~2.5:1 mixture of rotamers) δ 6.46 (br s, 0.3H), 5.06 (br s, 0.7H), 4.00 (br s, 1.4H), 3.93 (br s, 0.6H), 1.49 (s, 9H);

^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 174.6 (173.9), 156.0, 80.4, 42.2 (d, *J* = 63.3 Hz), 28.3 (29.7);

HRMS (ESI): calcd. for $C_6[^{13}C]H_{12}NO_4$ $[M-H]^-$: 175.0805. Found 175.0803.

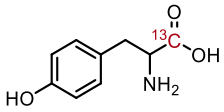


3-16 The preformed imine carboxylate salt was prepared according to the general procedure E from ^{12}C-serine (10.5 mg, 0.10 mmol, 1.0 equiv.), benzaldehyde (10.6 mg, 0.10 mmol, 1.0 equiv.), and Cs_2CO_3 (16.3 mg, 0.50 mmol, 0.50 equiv.) in MeOH (0.5 mL). The reaction mixture was allowed to stir at 70 ˚C for 1 h. After the solvent swap, the carboxylate imine salt was subjected to the exchange conditions (General Procedure E) in DMSO (1.0 mL) to prepare the labeled amino acid. The reaction mixture was allowed to stir at 90 ˚C for 1 h. ^{1}H NMR yield: 50%. The crude reaction mixture was not subjected to Boc_2O protection, it was cooled to room temperature, diluted with H_2O (10 mL), and acidified to pH 1–2, using 1M HCl (2–3 mL). Then, the crude mixture was concentrated in vacuo and subsequently lyophilized to get rid of the DMSO. Isolated in 41% yield, 27% ^{13}C incorporation (HRMS) as a white solid after purification by preparative-HPLC (Agilent Prep-C18 column, 1% MeOH in H_2O, 30 mL/min). Note: The product contains trace amounts of DSS internal standard.

^{1}H NMR (D_2O, 500 MHz) δ 4.19 (t, *J* = 3.9 Hz, 1H), 4.08 (dd, *J* = 12.7, 4.3 Hz, 1H), 3.99 (dd, *J* = 12.7, 4.3 Hz, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 171.2, 60.3, 55.8;

HRMS (ESI): calcd. for $C_2[^{13}C]H_6NO_3$ $[M-H]^-$: 105.0387. Found 105.0389.

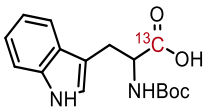


3-17 Prepared according to the general procedure A from ^{12}C-tyrosine (36.2 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 48 h. ^{1}H NMR yield: 78%. The crude reaction mixture was not subjected to Boc_2O protection, it was cooled to room temperature, diluted with H_2O (10 mL), and acidified to pH 1–2, using 1 M HCl (2–3 mL). Then, the crude mixture was concentrated in vacuo and subsequently lyophilized to get rid of the DMSO. Isolated in 65% yield, 75% ^{13}C incorporation (HRMS) as a white solid after purification by preparative-HPLC (Agilent Prep-C18 column, 1% MeOH in H_2O, 8.0 mL/min).

^{1}H NMR (D_2O, 500 MHz) δ 7.29–7.24 (m, 2H), 6.99–6.94 (m, 2H), 4.36–4.31 (m, 1H), 3.37–3.31 (m, 1H), 3.25–3.19 (m, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 172.7, 156.1, 131.8, 126.7, 116.9, 55.4 (d, J = 59.3 Hz), 35.8;

HRMS (ESI): calcd. for $C_8[^{13}C]H_{10}NO_3$ $[M-H]^-$: 181.0700. Found 181.0697.

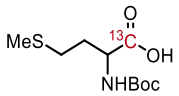


3-18 Prepared according to the general procedure A from ^{12}C-tryptophan (20.4 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (2.7 mg, 0.02 mmol, 0.20 equiv.), and Cs_2CO_3 (13.0 mg, 0.04 mmol, 0.40 equiv.) in DMSO (1.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 93%. Then, the reaction mixture was diluted with H_2O (10 mL) and acidified to pH 1–2, using 1M HCl (2–3 mL). Then, the aqueous layer was extracted with EtOAc (5 × 10 mL). Next, the combined organics were washed with H_2O (1 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Isolated in 75% yield, 31% ^{13}C incorporation (HRMS) as a white solid after the workup step. No silica gel chromatography was necessary for purification.

^{1}H NMR (d_6-DMSO, 500 MHz) (~6:1 mixture of rotamers) δ 7.51 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.16 (br s, 0.2H), 7.15–7.11 (m, 0.8H), 7.08–7.03 (m, 1H), 7.00–6.90 (m, 1.8H), 6.54 (br d, *J* = 8.9 Hz, 0.2H), 4.17–4.10 (m, 0.8H), 4.09–4.04 (br s, 0.2H), 3.12 (dd, *J* = 15.1, 4.9 Hz, 1H), 2.96 (dd, *J* = 15.1, 9.0 Hz, 0.9H), 2.91–2.86 (m, 0.1H), 1.32 (s, 7.5H), 1.20 (s, 1.5H);

^{13}C NMR (d_6-DMSO, 125 MHz) (rotamers) δ 173.9 (174.1), 155.3 (154.5), 136.0, 127.1, 123.6 (123.9), 120.8, 118.3, 118.1, 111.3, 110.1, 78.0 (78.2), 54.5 (d, *J* = 61.7 Hz), 28.1 (27.7), 26.8;

HRMS (ESI): calcd. for $C_{15}[^{13}C]H_{19}N_2O_4$ $[M-H]^-$: 304.1384. Found 304.1382.

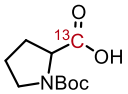


3-19 Prepared according to the general procedure A from ^{12}C-methionine (14.9 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (2.7 mg, 0.02 mmol, 0.20 equiv.), and Cs_2CO_3 (13.0 mg, 0.04 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 80%. Isolated in 68% yield, 36% ^{13}C incorporation (HRMS) as a white solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~3:1 mixture of rotamers) δ 6.20 (br s, 0.2H), 5.18 (br d, *J* = 8.2 Hz, 0.8H), 4.50–4.41 (br s, 0.8H), 4.36 (br s, 0.2H), 2.61 (t, *J* = 7.0 Hz, 2H), 2.2–2.16 (m, 1H), 2.13 (s, 3H), 2.05–1.95 (m, 1H), 1.48 (s, 9H);

^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 176.4 (176.2), 155.7, 80.5, 52.7, (d, *J* = 61.3 Hz), 31.7, 30.0, 28.3, 15.4;

HRMS (ESI): calcd. for $C_9[^{13}C]H_{18}NO_4S$ $[M-H]^-$: 249.0996. Found 249.0996.



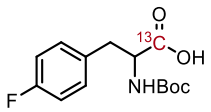
3-20 Prepared according to the general procedure A from ^{12}C-proline (23.0 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (20.4 mg, 0.15 mmol, 0.75 equiv.), and Cs_2CO_3 (65.2 mg, 0.20 mmol, 1.0 equiv.) in DMSO (2.0 mL). The reaction mixture

was allowed to stir for 48 h. ^{1}H NMR yield: 72%. Isolated in 62% yield, 9% ^{13}C incorporation (HRMS) as an off-white oil after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~2:1 mixture of rotamers) δ 4.37 (br d, *J* = 7.7 Hz, 0.7H), 4.28 (br s, 0.3H), 3.62–3.33 (m, 2H), 2.44–2.34 (m, 0.7H), 2.33–2.24 (m, 0.3H), 2.13–2.02 (m, 1H), 2.01–1.87 (m, 2H), 1.51 (s, 5.8H), 1.45 (s, 3.2H);

^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 173.9 (178.2), 157.0 (153.8), 81.8 (80.3), 59.4 (58.9), 47.1 (46.4), 30.9 (29.7), 28.4 (28.2), 24.4 (23.7);

HRMS (ESI): calcd. for $C_9[^{13}C]H_{16}NO_4$ [M-H]$^-$: 215.1118. Found 215.1117.



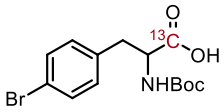
3-21 (±)-[C_1-^{13}C]Boc-4-fluorophenylalanine prepared according to the general procedure A from L-4-fluorophenylalanine (36.6 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 80%. Isolated in 55% yield (31.3 mg), 50% ^{13}C incorporation (HRMS) as a white solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~5:2 mixture of rotamers) δ 7.19–7.11 (m, 2H), 7.02–6.94 (m, 2H), 6.28 (br s, 0.3H), 4.96 (br s, 0.7H), 4.60 (br s, 0.7H), 4.36 (br s, 0.3H), 3.17 (dd, *J* = 14.6, 4.2 Hz, 1H), 3.04 (dd, *J* = 14.6, 9.1 Hz, 0.7H), 2.90 (br s, 0.3H), 1.42 (s, 6.5H), 1.35 (br s, 2.5H);

^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 176.4 (175.9), 162.1 (d, *J* = 260 Hz), 155.3 (156.3), 131.5 (132.0), 130.9 (d, *J* = 5.0 Hz), 115.5 (d, *J* = 19.8 Hz), 80.5 (81.8), 54.3 (d, *J* = 59.4 Hz) (56.0), 37.1 (38.1), 28.3 (28.1);

^{19}F NMR ($CDCl_3$, 376 MHz) (rotamers) δ –115.7 (–115.8);

HRMS (ESI): calcd. for $C_{13}[^{13}C]H_{17}FNO_4$ [M-H]$^-$: 283.1181. Found 283.1178.

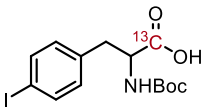


3-22 (±)-[C_1-^{13}C]Boc-4-bromophenylalanine prepared according to the general procedure A from L-4-bromophenylalanine (48.8 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 72%. Isolated in 60% yield (41.4 mg), 83% ^{13}C incorporation (HRMS) as an orange solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR (d_4-MeOH, 500 MHz) (~4:1 mixture of rotamers) δ 7.41 (d, *J* = 8.5 Hz, 2H), 7.15 (d, *J* = 8.5 Hz, 2H), 4.32 (m, 0.8H), 4.24 (br s, 0.2H), 3.13 (ddd, *J* = 13.7, 5.1, 2.6 Hz, 1H), 2.86 (ddd, *J* = 13.7, 8.4, 2.6 Hz, 1H), 1.37 (s, 7H), 1.33 (s, 2H);

^{13}C NMR (d_4-MeOH, 125 MHz) (rotamers) δ 175.1, 157.8, 138.0, 132.4, 132.3, 121.5, 80.6 (81.5), 56.1 (d, *J* = 59.3 Hz) (57.2), 38.2 (38.6), 28.7 (28.4);

HRMS (ESI): calcd. For $C_{13}[^{13}C]H_{17}NO_4[^{79}Br]$ $[M-H]^-$: 343.0380. Found 343.0379.



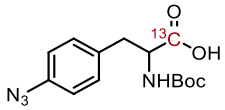
3-23 (±)-[C_1-^{13}C]Boc-4-iodophenylalanine prepared according to the general procedure A from L-4-iodophenylalanine (58.2 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 75%. Isolated in 63% yield (49.5 mg), 84% ^{13}C incorporation (HRMS) as a white solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~3:1 mixture of rotamers) δ 7.62 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.18 (br s, 0.2H), 4.95 (br s, 0.7H), 4.58 (br s, 0.8H), 4.36 (br

s, 0.3H), 3.14 (dd, J = 12.6, 6.0 Hz, 1H), 3.00 (dd, J = 12.6, 8.2 Hz, 0.7H), 2.85 (br s, 0.3H), 1.42 (s, 7H), 1.34 (br s, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 175.9 (175.6), 155.3 (156.1), 137.7, 135.5, 131.4, 92.7, 80.5 (81.8), 54.1 (d, J = 57.8 Hz) (55.1), 37.4 (38.6), 28.3 (28.1);

HRMS (ESI): calcd. for $C_{13}[^{13}C]H_{17}INO_4$ $[M-H]^-$: 391.0241. Found 391.0237.

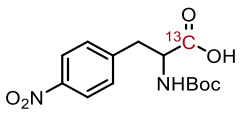


3-24 (±)-[C_1-^{13}C]Boc-4-azidophenylalanine prepared according to the general procedure A from L-4-azidophenylalanine (41.2 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 93%. Isolated in 65% yield (40.0 mg), 56% ^{13}C incorporation (HRMS) as a yellow solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR (d_6-Acetone, 500 MHz) (~7:1 mixture of rotamers) δ 7.43 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 6.13 (br d, J = 8.0 Hz, 0.8H), 5.80 (br s, 0.1H), 4.50 (m, 0.9H), 4.41 (br s, 0.2H), 3.30 (dd, J = 14.5, 4.7 Hz), 3.09 (dd, J = 14.5 Hz, 9.8 Hz, 1H), 1.45 (s, 9H);

^{13}C NMR (d_6-Acetone, 125 MHz) (rotamers) δ 173.4, 156.3, 139.2, 135.6, 131.8, 119.8, 79.4 (80.0), 55.7 (d, J = 60.9 Hz) (57.1), 37.6 (38.2), 28.6 (28.4);

HRMS (ESI): calcd. for $C_{13}[^{13}C]H_{17}N_4O_4$ $[M-H]^-$. 306.1289. Found 306.1284.



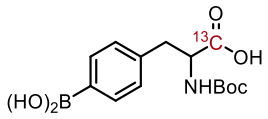
3-25 (±)-[C_1-^{13}C]Boc-4-nitrophenylalanine prepared according to the general procedure A from L-4-nitrophenylalanine (0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (42.0 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for

28 h. ^{1}H NMR yield: 76%. Isolated in 58% yield (36.0 mg), 48% ^{13}C incorporation (HRMS) as an orange solid after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR (d_6-Acetone, 500 MHz) (~7:1 mixture of rotamers) δ 8.28 (d, *J* = 8.7 Hz, 2H), 7.69 (d, *J* = 8.7 Hz, 2H), 6.32 (br d, *J* = 9.5 Hz, 0.7 H), 5.95 (br s, 0.1H), 4.61 (m, 0.9H), 4.52 (br s, 0.1H), 3.47 (dd, *J* = 14.4, 5.8 Hz, 1H), 3.26 (dd, *J* = 14.4, 9.5 Hz), 1.43 (s, 8.4H), 1.42 (br s, 0.6H);

^{13}C NMR (d_6-Acetone, 125 MHz) (rotamers) δ 173.1, 156.3, 147.9, 146.9, 131.5, 124.1, 79.5, 55.3 (d, *J* = 60.1 Hz) (56.8), 38.1 (38.6), 28.5;

HRMS (ESI): calcd. for $C_{13}[^{13}C]H_{17}N_2O_6$ $[M-H]^-$: 310.1126. Found 310.1129.

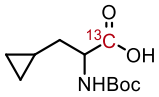


3-26 (±)-[C_1-^{13}C]Boc-4-boronophenylalanine prepared according to the general procedure A from L-4-boronophenylalanine (41.8 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 39%. Isolated in 32% yield (19.9 mg), 43% ^{13}C incorporation (HRMS) as an off-white solid after purification by silica gel chromatography (3% to 7% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR (d_4-MeOH, 700 MHz) (~4:1 mixture of rotamers) δ 7.66 (br s, 0.4H), 7.53 (br d, *J* = 7.6 Hz, 1.6H), 7.24 (br d, J = 7.6 Hz, 1.6H), 7.22–7.17 (m, 0.4H), 4.36–4.30 (m, 0.8H), 4.25 (br s, 0.2H), 3.17 (dd, *J* = 13.3, 4.7 Hz, 1H), 2.92 (dd, *J* = 13.3, 8.6 Hz, 0.8H), 2.87–2.81 (m, 0.2H), 1.38 (s, 7.1H), 1.31 (s, 1.9H);

^{13}C NMR (d_4-MeOH, 175 MHz) (rotamers) δ 176.1 (176.4), 157.7, 140.5, 134.6, 129.7, 80.4, 56.6 (d, *J* = 56.6 Hz), 39.0, 28.7 (28.4);

HRMS (ESI): calcd. for $C_{13}[^{13}C]H_{19}[^{11}B]NO_6$ $[M-H]^-$: 309.1344. Found 309.1337.

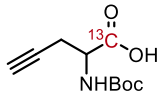


3-27 (±)-[C_1-^{13}C]Boc-cyclopropylalanine prepared according to the general procedure A from L-cyclopropylalanine (25.8 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 85%. Isolated in 68% yield (31.2 mg), 42% ^{13}C incorporation (HRMS) as a colorless oil after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~3:1 mixture of rotamers) δ 6.09 (br s, 0.2H), 5.17 (br d, J = 7.3 Hz, 0.7H), 4.40 (br s, 0.8H), 4.20 (br s, 0.3H), 1.75–1.65 (m, 2H), 1.43 (s, 9H), 0.82–0.70 (m, 1H), 0.54–0.44 (m, 2H), 0.18–0.07 (m, 2H);

^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 177.6, 155.5 (156.3), 80.2 (81.4), 53.9 (d, J = 59.7 Hz) (55.2), 37.0 (37.3), 28.3, 7.0 (7.1), 4.3 (4.2);

HRMS (ESI): calcd. for $C_{10}[^{13}C]H_{18}NO_4$ $[M-H]^-$: 229.1275. Found 229.1268.



3-28 (±)-[C_1-^{13}C]Boc-propargylalanine prepared according to the general procedure A from L-propargylalanine (22.6 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 80%. Isolated in 69% yield (29.6 mg), 61% ^{13}C incorporation (HRMS) as a yellow oil after purification by silica gel chromatography (1% to 3% MeOH in DCM with 0.1% AcOH). The AcOH was removed by chasing the product with toluene three times on a rotovap.

^{1}H NMR ($CDCl_3$, 500 MHz) (~4:1 mixture of rotamers) δ 6.07 (br s, 0.1H), 5.37 (br d, J = 8.2 Hz 0.9H), 4.60–4.50 (m, 0.8H), 4.34 (br s, 0.2H), 2.88–2.74 (m, 2H), 2.11 (t, J = 2.5 Hz, 0.9H), 2.05 (t, J = 2.5 Hz, 0.1H), 1.49 (s, 9H);

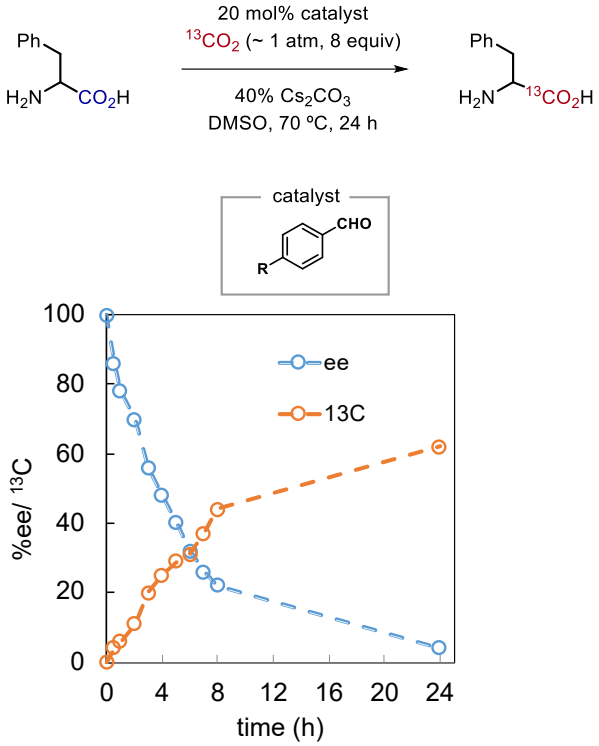
^{13}C NMR ($CDCl_3$, 125 MHz) (rotamers) δ 175.2, 155.4 (155.6), 80.7, 78.2, 72.0, 67.4, 51.8 (d, J = 60.8 Hz), 28.3, 22.5;

HRMS (ESI): calcd. for $C_9[^{13}C]H_{14}NO_4$ $[M-H]^-$: 213.0962. Found 213.0961.

Chiral Resolution of Amino Acids by DKR, KR and Prep-HPLC

a) Racemization of Enantiopure Phenylalanine

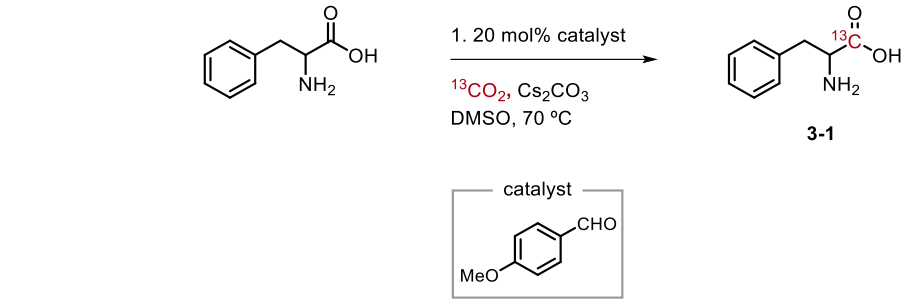
Isotopic labeling occurs with racemization of chiral enantiopure α-amino acids, as shown in the plot below. Note that sampling the reaction multiple times leads to a slight decrease in the final ^{13}C incorporation compared to standard conditions.



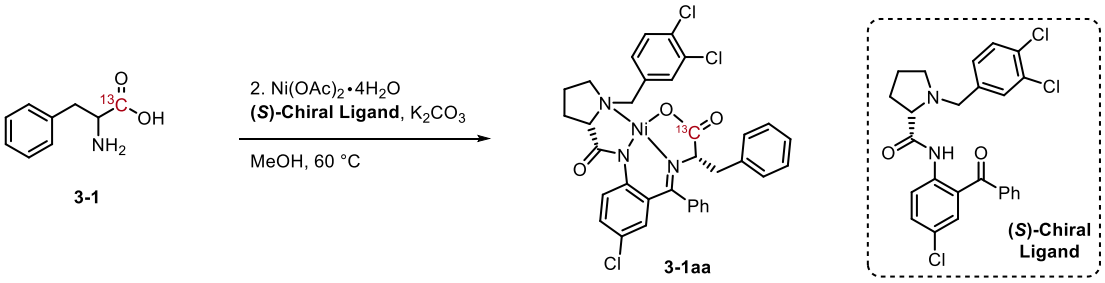
Note: the initial carboxylate exchange step to prepare enantiopure α-amino acids can be performed, using either the general procedure A or the General procedure E.

b) L-phenylalanine (3-1a)

1. DKR, Using Chiral Nickel Complexation/Decomplexation, 3 Steps

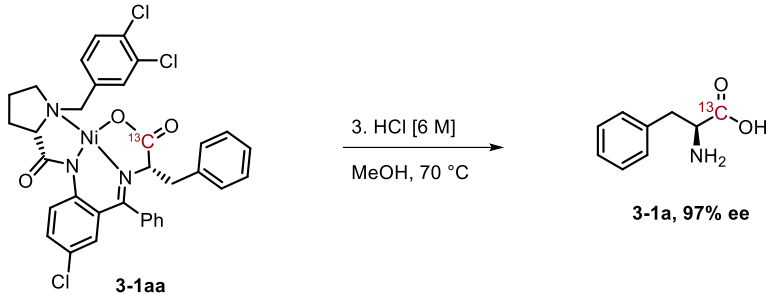


3-1 (±)-[C_1-^{13}C]phenylalanine *Step 1*. Prepared according to the general procedure A from (±)-phenylalanine (33.0 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 26 h. The crude reaction mixture was lyophilized to remove the DMSO and subsequently used directly in the next step without further purification. The isolated yield was assumed to be quantitative.



3-1aa *Step 2*. Adapted from a literature procedure.[171] In an atmosphere-controlled glovebox, **3-1** (0.20 mmol, assumed quantitative yield in *Step 1*), (*S*)-chiral ligand (88.3 mg, 0.18 mmol, 1.0 equiv.), $Ni(OAc)_2 \cdot 4H_2O$ (49.8 mg, 0.20 mmol, 1.1 equiv.), K_2CO_3 (124 mg, 0.90 mmol, 5.0 equiv.), and MeOH (3.7 mL) were added sequentially to an 8-dram vial charged with a stir bar. The vial was sealed with a PTFE-lined cap, removed from the glovebox, and stirred subsequently at 60 ˚C in an aluminum block. Upon completion of the reaction (3 h), the vial was cooled to room temperature. The reaction mixture was quenched with ice cold AcOH (5% in H_2O, 20 mL), and the aqueous layer was extracted with DCM (3 × 10 mL). The combined

organics were washed with brine (1 × 10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was used directly in the next step without further purification. The isolated yield was assumed to be quantitative.



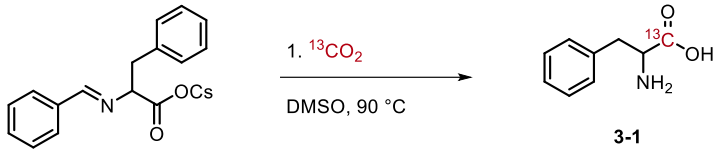
3-1a L-[C_1-^{13}C]phenylalanine *Step 3*. Adapted from a literature procedure.[178] In an 8-dram vial charged with a stir bar was added **3-1aa** (0.18 mmol, assumed quantitative yield in *Step 2*) and MeOH (3.6 mL). The solution was stirred vigorously at room temperature, and 6 M HCl (1.0 mL) was added dropwise while stirring. The reaction was then heated to 70 ˚C in an aluminum block. Upon completion of the reaction (0.5 h), the vial was cooled to room temperature and concentrated in vacuo. The pH was adjusted carefully to ~9 with concentrated NH_4OH (~0.5 mL). Then, the aqueous layer was extracted with DCM (5 × 10 mL) to wash away the chiral ligand. The chiral ligand can be recovered from the combined organics for future use. Next, the aqueous layer was acidified to pH ~3 with 3M HCl (~2 mL). The aqueous layer was lyophilized subsequently to remove the water. Isolated in 70% yield over 3 steps (23.2 mg), 63% ^{13}C incorporation (HRMS), 97% *ee* after purification by preparative-HPLC (Agilent Prep-C18 column, 5% MeOH in H_2O, 30 mL/min) as a white solid.

^{1}H NMR (D_2O, 500 MHz) δ 7.42–7.38 (m, 2H), 7.37–7.32 (m, 1H), 7.31–7.28 (m, 2H), 3.99–3.94 (m, 1H), 3.30 – 3.23 (m, 1H), 3.09 (dd, J = 14.5, 8.1 Hz, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 173.6, 135.0, 129.4, 129.2, 127.8, 56.0 (d, J = 56.2 Hz), 36.3;**HRMS (ESI):** calcd. for C_8[^{13}C]$H_{10}NO_2$ [M-H]$^-$: 165.0751. Found 165.0749;

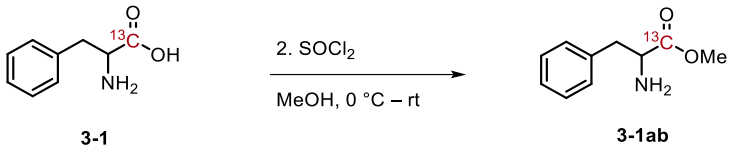
Chiral HPLC: Astec Chirobiotic-T column (30% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 4.4 min (major), t_r = 5.5 min (minor).

2. KR, Using α-Chymotrypsin, 3 Steps



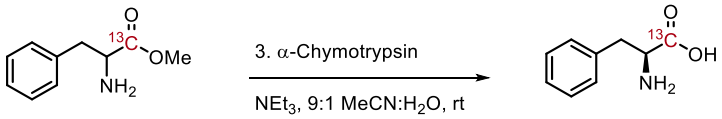
3-1

3-1 (±)-[C_1-^{13}C]phenylalanine *Step 1*. Note: three 0.2 mmol scale carboxylate exchange reactions were run in parallel, and the crude reaction mixtures were combined before performing *Step 2*. The preformed imine carboxylate salt was prepared according to the General Procedure E from (±)-phenylalanine (33.1 mg, 0.20 mmol, 1.0 equiv.), benzaldehyde (21.2 mg, 0.20 mmol, 1.0 equiv.), and Cs_2CO_3 (32.6 mg, 0.10 mmol, 0.50 equiv.) in MeOH (1.0 mL). The reaction mixture was allowed to stir at 70 ˚C for 1 h. After the MeOH to DMSO (1 mL) solvent swap, the carboxylate imine salt was subjected to the exchange conditions (general procedure E) to prepare the labeled amino acid. The reaction mixture was allowed to stir at 90 ˚C for 1 h. The crude reaction mixture was lyophilized to remove the DMSO. Then, the mixture was acidified to pH 1 –2 with 1M HCl (2–3 mL), and the aqueous layer was washed with EtOAc (3 × 10 mL). The aqueous layer was lyophilized to remove the H_2O, and the product was used directly in the next step without further purification. The isolated yield was assumed to be quantitative.



3-1 **3-1ab**

3-1ab *Step 2*. Adapted from a literature procedure.[179] To a 1-dram vial charged with a stir bar was added **3-1** (0.60 mmol, assumed quantitative yield in *Step 1*) and anhydrous MeOH (0.84 mL). The resulting mixture was cooled to 0 ˚C, after which thionyl chloride (0.90 mmol, 1.5 equiv., 60.0 µL) was slowly added. The reaction was allowed to slowly warm up to room temperature. After stirring for 20 h, the crude mixture was concentrated in vacuo. The crude product was used directly in the next step without further purification, 70% calibrated ^{1}H NMR yield over two steps.



3-1ab **3-1a, 99% ee**

3-1a L-[C_1-^{13}C]-phenylalanine *Step 3*. Adapted from a literature procedure.[169] In an 8-dram vial charged with a stir bar was added **3-1ab** (0.42 mmol, based on two step calibrated ^{1}H NMR yield from *Steps 1* and *2*) and MeCN (8.4 mL). This was followed by the addition of triethylamine (58.3 µL, 0.42 mmol, 1.0 equiv.) and a solution of α-chymotrypsin from bovine pancreas (2.8 mg, ≥40 units/mg protein) in H_2O (0.93 mL). Then, the reaction mixture was stirred at room temperature for 24 h, and concentrated in vacuo. The aqueous layer was lyophilized subsequently to remove the water. Isolated in 26% yield over 3 steps (26.0 mg), 86% ^{13}C incorporation (HRMS), 99% *ee* after purification by preparative-HPLC (Agilent Prep-C18 column, 5% MeOH in H_2O, 30 mL/min) as a white solid.

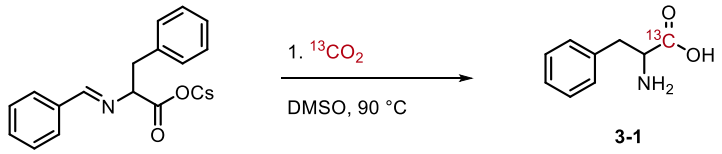
^{1}H NMR (D_2O, 500 MHz) δ 7.42–7.38 (m, 2H), 7.37–7.32 (m, 1H), 7.31–7.28 (m, 2H), 3.99–3.94 (m, 1H), 3.30–3.23 (m, 1H), 3.09 (dd, J = 14.5, 8.1 Hz, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 173.6, 135.0, 129.4, 129.2, 127.8, 56.0 (d, J = 56.2 Hz), 36.3;

HRMS (ESI): calcd. for $C_8[^{13}C]H_{10}NO_2$ $[M-H]^-$: 165.0751. Found 165.0748;

Chiral HPLC: Astec Chirobiotic-T column (30% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 4.3 min (major), t_r = 5.4 min (minor).

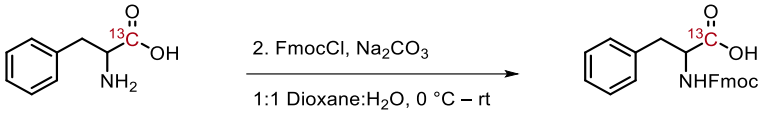
3. Resolution of Fmoc-Phenylalanine by Preparative-HPLC, 2 Steps



3-1

3-1 (±)-[C_1-^{13}C]phenylalanine *Step 1*. The preformed imine carboxylate salt was prepared according to the general procedure E from (±)-phenylalanine (33.1 mg, 0.20 mmol, 1.0 equiv.), benzaldehyde (21.2 mg, 0.20 mmol, 1.0 equiv.), and Cs_2CO_3 (32.6 mg, 0.10 mmol, 0.50 equiv.) in MeOH (1.0 mL). The reaction mixture was allowed to stir at 70 ˚C for 1 h. After the solvent swap, the carboxylate imine salt was

subjected to the exchange conditions (general procedure E) in DMSO (1.0 mL) to prepare the labeled amino acid. The reaction mixture was allowed to stir at 90 ˚C for 1 h. The crude reaction mixture was lyophilized to remove the DMSO. Then, the mixture was acidified to pH 1–2 with 1M HCl (2–3 mL), and the aqueous layer was washed with EtOAc (3 × 10 mL). The aqueous layer was lyophilized to remove the H_2O, and the product subsequently was used directly in the next step without further purification. The isolated yield was assumed to be quantitative.



3-1 **3-1b**

3-1b (±)-[C_1-^{13}C]Fmoc-phenylalanine *Step 2*. To a 1-dram vial charged with a stir bar was added **3-1** (0.20 mmol, assumed quantitative yield in *Step 1*), 1:1 Dioxane:H_2O (1.3 mL), and Na_2CO_3 (116.7 mg, 1.1 mmol, 5.5 equiv.). Then, the reaction mixture was cooled to 0 °C. FmocCl (62.1 mg, 0.24 mmol, 1.2 equiv.) was added at 0 °C, and the reaction was allowed to warm up to room temperature. After overnight stirring (16 h), the reaction mixture was acidified, using 1M HCl (5 mL), and the aqueous layer was extracted with DCM (3 × 20 mL). The combined organics were washed with water (1 × 10 mL) and brine (1 × 10 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. Isolated in 56% yield (43.5 mg), 87% ^{13}C incorporation (HRMS) as a white solid after purification by silica gel chromatography (0 to 4% MeOH in DCM with 0.1% AcOH).

Chiral Resolution of 3-1b by preparative-HPLC: Resolution of **3-1b** was carried out by using a Phenomenex Lux i-Amylose-3 column (10 × 250 mm, 5 µm particle size) under normal phase conditions, 20% DCM in hexanes with 1% AcOH, and 1% EtOH, 5 mL/min. Racemic material was loaded on the column at a concentration of 10 mg/mL in 30% DCM in hexanes with 3% EtOH and 1% AcOH. Repeated injections of 300 µL were performed. Five injections gave a 27% isolated yield (21.0 mg) of D-**3-1b** as a white solid, >99% ee, and a 24% isolated yield (18.6 mg) of L-**3-1b** as a white solid, 96% *ee*.

^{1}H NMR ($CDCl_3$, 500 MHz) (~4:1 mixture of rotamers) δ 7.79 (d, *J* = 7.7 Hz, 2H), 7.63–7.50 (m, 2H), 7.42 (t, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 7.8 Hz, 4H), 7.17 (d, *J* = 7.0 Hz, 1.7H), 7.09–7.06 (m, 0.3H), 5.66 (br s, 0.2 H), 5.22 (d, *J* = 8.2 Hz, 0.8 H), 4.75 (q, *J* = 6.3 Hz, 0.8H), 4.48 (dd,~*J* = 10.5, 7.2 Hz, 1H), 4.40 (dd, *J* = 10.5, 7.0 Hz, 1H), 4.23 (t, *J* = 7.1 Hz, 1H), 3.25 (dd, *J* = 13.9, 5.4 Hz, 0.8H), 3.15 (dd, *J* = 13.9, 5.4 Hz, 0.8H), 3.08–2.95 (m, 0.2H), 2.94–2.80 (m, 0.2H);

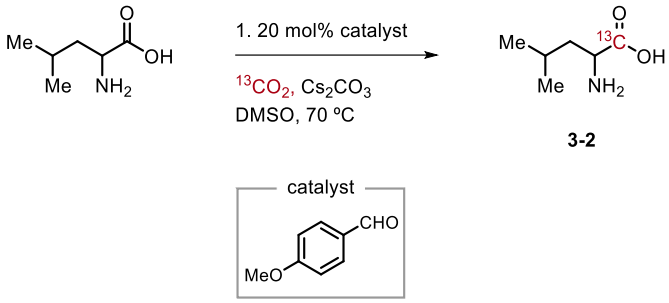
^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.7, 155.9, 143.7, 141.4, 135.7, 129.4, 128.7, 127.8, 127.3, 127.1, 125.1, 120.0, 67.1, 47.1, 37.8, 39.8;

HRMS (ESI): calcd. for $C_{23}[^{13}C]H_{20}NO_4$ $[M-H]^-$: 387.1431. Found 387.1427.

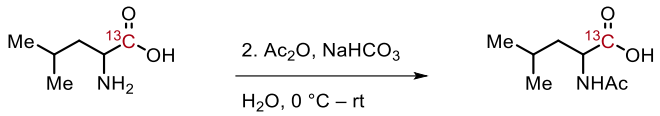
Chiral HPLC: ChiralPak IC column (10% IPA in Hexanes with 0.1% AcOH, 1.5 mL/min), t_r = 8.1 min (L-enantiomer), t_r = 10.2 min (D-enantiomer).

b) L-Leucine (3-2a)

1. KR, Using Acylase I from *Aspergillus Melleus*, 3 steps

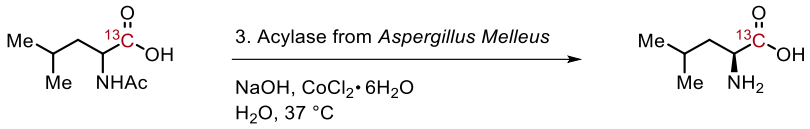


3-2 (±)-[C_1-^{13}C]-leucine *Step 1*. Note: three 0.2 mmol scale reactions were run in parallel, and the crude reaction mixtures were combined before performing *Step* 2. Prepared according to the general procedure A from L-leucine (26.2 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction mixture was allowed to stir for 28 h. The crude reaction mixture was lyophilized to remove the DMSO, and subsequently used directly in the next step without further purification. The isolated yield was assumed to be quantitative.



3-2 **3-2aa**

3-2aa *Step 2*. Adapted from a literature procedure.[180] To a 1-dram vial charged with a stir bar was added **3-2a** (0.60 mmol, assumed quantitative yield in *Step 1*), followed by a 5% aqueous $NaHCO_3$ solution (1.68 mL). The reaction mixture was cooled to 0 °C, after which acetic anhydride (206 µL, 1.8 mmol, 3.0 equiv.) was added dropwise over a period of 30 min. The resulting mixture was allowed to warm up to room temperature, and subsequently stirred for 18 h. The crude mixture was then acidified to pH 2–3 with 6M HCl and then lyophilized to remove the H_2O. The crude product was used directly in the next step without further purification. The isolated yield was assumed to be quantitative.



3-2aa **3-2a, >99% ee**

3-2a L-[C_1-^{13}C]-leucine *Step 3*. Adapted from a literature procedure.[170] In an 8-dram vial charged with a stir bar was added sequentially **3-2aa** (0.6 mmol, assumed quantitative yield in *Step 2*), NaOH pellets (26.0 mg, 0.66 mmol, 1.1 equiv.), and H_2O (1.04 mL). Next, $CoCl_2$●$6H_2O$ (300 µg, 1.2 µmol, 0.002 equiv.) was added, the colourless solution was adjusted to pH 7.7–7.9 with 2M HCl, and subsequently was heated to 37 °C. Then, acylase from *Aspergillus Melleus* (47.0 mg, >0.5 units/mg) was added, and the reaction was stirred at 37 °C for 48 h. The pH was monitored carefully and kept at 7.9 with 1M NaOH by testing with pH strips. After 48 h, the resultant heterogeneous mixture was acidified with 2M HCl. The aqueous layer was washed with $CHCl_3$ (5 × 10 mL) to remove the *N*-acetyl D-leucine, and the aqueous layer was lyophilized to remove the water. Isolated in 38% yield over 3 steps (30.1 mg), 38% ^{13}C incorporation (HRMS), >99% *ee* as a white solid.

^{1}H NMR (D_2O, 500 MHz) δ 4.03–3.97 (m, 1H), 1.87–1.68 (m, 3H), 0.95 (app t, J = 5.9 Hz, 6H);

^{13}C NMR (D_2O, 125 MHz) δ 174.3, 52.9 (d, J = 60.2 Hz), 40.0, 25.0, 22.7, 21.9;

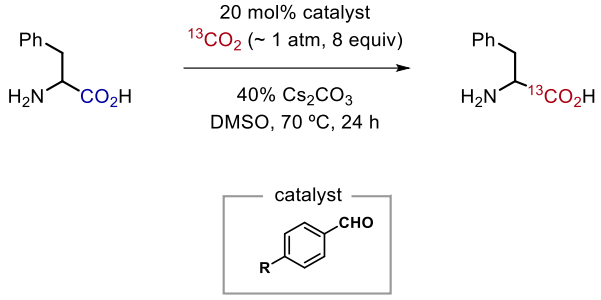
HRMS (ESI): calcd. for $C_5[^{13}C]H_{14}NO_2$ $[M+H]^+$: 133.1053. Found 133.1054;

Chiral HPLC: Astec Chirobiotic-T column (40% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 2.3 min (major), t_r = 2.7 min (minor).

Mechanistic Analysis

a) Kinetic Analysis and Aldehyde Catalyst Lifetime Studies

1. Kinetic Analysis of Aldehyde Catalysts for the Carboxylate Exchange of Phenylalanine

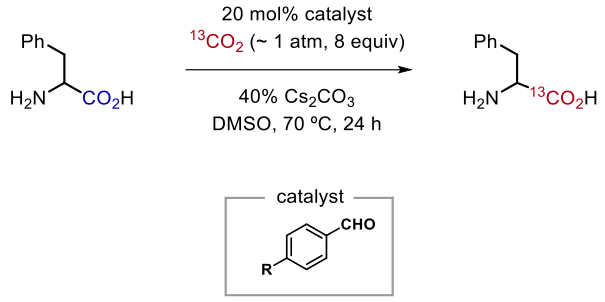


time (h)	^{13}C inc. (%) R= OMe	^{13}C inc. (%) R= H	^{13}C inc. (%) R= CF_3	^{13}C inc. (%) R= CN
1 h	7	15	21	16
2 h	10	24	27	18
3 h	15	30	29	18
4 h	17	30	29	19
6 h	25	36	30	19
8 h	27	41	30	19
10 h	33	42	31	20
24 h	45	48	33	24

The experiment followed the general procedure A, using (±)-phenylalanine (16.5 mg, 0.10 mmol, 1.0 equiv.) with different catalysts, 4-methoxybenzaldehyde (2.7 mg, 0.02 mmol, 0.20 equiv.), benzaldehyde (2.1 mg, 0.02 mmol, 0.20 equiv.), 4-cyanobenzaldehyde (2.6 mg, 0.02 mmol, 0.20 equiv.), 4-CF_3-benzaldehyde (3.5 mg, 0.02 mmol, 0.20 equiv.), Cs_2CO_3 (13 mg, 0.04 mmol, 0.40 equiv.), and DSS internal standard in DMSO (1.0 mL). The reaction mixtures were sampled at 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, and 24 h. To determine the percent recovery of the amino acid, a small

aliquot (~10 µL) of the reaction was placed in 0.70 mL D_2O for calibrated ^1H NMR analysis, using DSS as the reference signal. To determine the % ^{13}C incorporation, a small aliquot of the reaction (~5 µL) was placed in 1.0 mL of 1:1 MeOH:H_2O/0.1% HCO_2H for LC-MS analysis. Above is the representation of data for all catalysts at different time points. The terminal amounts of ^{13}C incorporation are reduced compared to standard experiments, likely because of [^{13}C]CO_2 loss during reaction sampling.

2. Lifetime of Aldehyde Catalysts for the Carboxylate Exchange of Phenylalanine

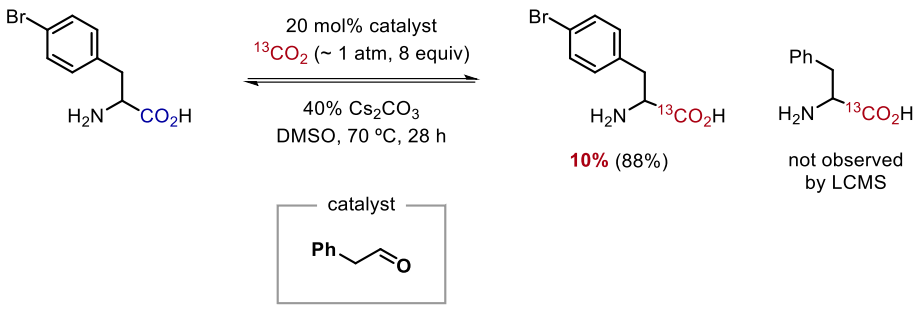


time (h)	Recovery(%) R= OMe	Recovery(%) R= H	Recovery(%) R= CF_3	Recovery(%) R= CN
0 h	18	15	18	11
1 h	12	11	3	1
6 h	11	5	0	1
24 h	2	0	0	0

The experiment followed the general procedure A, using (±)-phenylalanine (16.5 mg, 0.10 mmol, 1.0 equiv.) with different catalysts, 4-methoxybenzaldehyde (2.7 mg, 0.02 mmol, 0.20 equiv.), benzaldehyde (2.1 mg, 0.02 mmol, 0.20 equiv.), 4-cyanobenzaldehyde (2.6 mg, 0.02 mmol, 0.20 equiv.), 4-CF_3-benzaldehyde (3.5 mg, 0.02 mmol, 0.20 equiv.), Cs_2CO_3 (13 mg, 0.04 mmol, 0.40 equiv.), and TMB internal standard in DMSO (1.0 mL). The reaction mixtures were sampled at, 0 h, 1 h, 6 h, 24 h. To determine the percent recovery of the aldehyde, a small aliquot (~10 µL) of the reaction was placed in 0.90 mL $CDCl_3$, quenched with 1.0 M HCl, and passed through a pipet containing Na_2SO_4 into an NMR tube for calibrated ^1H NMR analysis, using

TMB as the reference signal. Above is the representation of data for all catalysts at different time points.

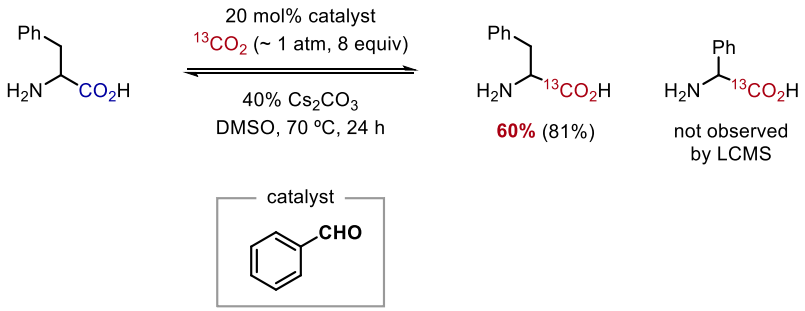
b) Specific Experimental Details for Crossover to Observe Phenylalanine



The experiment followed the general procedure A, using L-4-bromophenylalanine (24.4 mg, 0.10 mmol, 1.0 equiv.), phenylacetaldehyde (2.4 mg, 0.02 mmol, 0.20 equiv.), and Cs_2CO_3 (13 mg, 0.04 mmol, 0.40 equiv.) in DMSO (1.0 mL). The reaction mixture was allowed to stir for 28 h. ^{1}H NMR yield: 88%, ^{13}C incorporation: 10% (LCMS). $^{12/13}C$-labeled phenylalanine was not detected by LCMS. In a standard experiment, in which phenylalanine was added in 1% before analysis, it was detected readily. Thus, the smallest quantity of phenylalanine formed in a reaction that can be detected accurately by the LCMS protocol used is at least 1%.

c) Specific Experimental Details for Crossover to Observe Phenylglycine

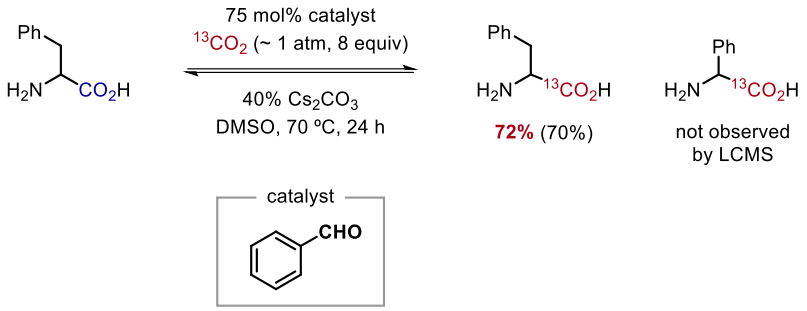
1. Standard Conditions, Using 20 mol % Benzaldehyde Catalyst



The experiment followed the general procedure A from (±)-phenylalanine (16.5 mg, 0.10 mmol, 1.0 equiv.) benzaldehyde (2.1 mg, 0.02 mmol, 0.20 equiv.), and Cs_2CO_3 (13 mg, 0.04 mmol, 0.40 equiv.) in DMSO (1.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 81%, ^{13}C incorporation: 60% (LCMS). $^{12/13}C$-

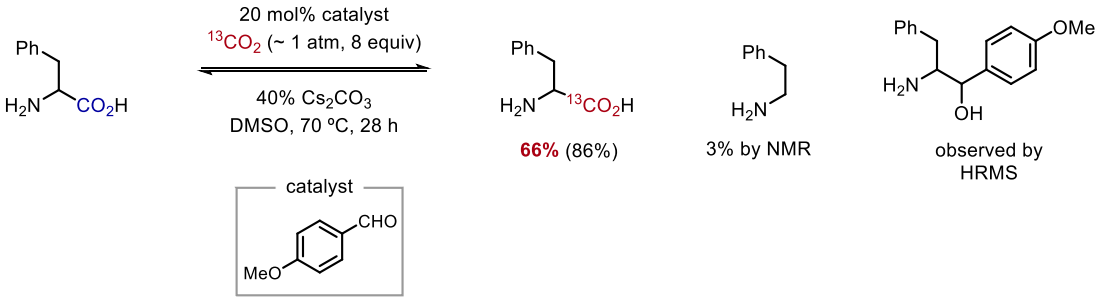
labeled phenylglycine was not detected by LCMS. In a standard experiment, in which phenylglycine was added in 1% before analysis, it was detected readily. Thus, the smallest quantity of phenylglycine formed in a reaction that can be accurately detected by the LCMS protocol used is at least 1%.

2. Standard Conditions, Using 75 mol % Benzaldehyde Catalyst



The experiment followed the general procedure A from (±)-phenylalanine (16.5 mg, 0.10 mmol, 1.0 equiv.) benzaldehyde (8.0 mg, 0.075 mmol, 0.75 equiv.) and Cs_2CO_3 (32.5 mg, 0.10 mmol, 1.0 equiv.) in DMSO (1.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 70%, ^{13}C incorporation 72% (LCMS). $^{12/13}C$-labeled phenylglycine was not detected by LCMS. In a standard experiment, in which phenylglycine was added in 1% before analysis, it was detected readily. Thus, the smallest quantity of phenylglycine formed in a reaction that can be detected accurately by the LCMS protocol used is at least 1%.

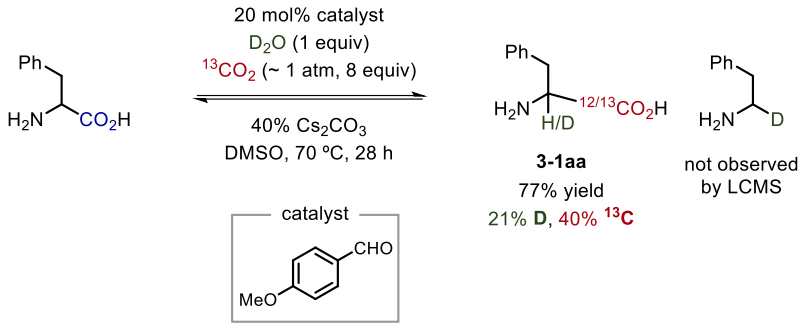
d) Detection of Side Products



The experiment followed the general procedure A from (±)-phenylalanine (33.0 mg, 0.20 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (5.4 mg, 0.04 mmol, 0.20 equiv.), and Cs_2CO_3 (26.1 mg, 0.08 mmol, 0.40 equiv.) in DMSO (2.0 mL). The reaction

mixture was allowed to stir for 28 h. ^{1}H NMR yield: 86%, ^{13}C incorporation: 66%. Protodecarboxylation product (phenethylamine) was observed by NMR (^{1}H NMR yield: 3%). The mass for the trapped aldehyde product was detected by HRMS. m/z = 258.1478 [M+H]. There was no aldehyde catalyst remaining at the end of the reaction.

e) Labeling Experiment with D_2O



3-1aa Prepared according to the general procedure A from (±)-phenylalanine (16.5 mg, 0.10 mmol, 1.0 equiv.), 4-methoxybenzaldehyde (2.7 mg, 0.02 mmol, 0.20 equiv.), and Cs_2CO_3 (13.0 mg, 0.04 mmol, 0.40 equiv.) and D_2O (1.8 mL, 0.10 mmol, 1.0 equiv.) in DMSO (1.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 77%, Isolated in 68% yield, 40% ^{13}C incorporation (HRMS), 21% ^{2}H incorporation (NMR) after purification by preparative-HPLC (Agilent Prep-C18 column, 5% MeOH in H_2O, 15 mL/min) as a white solid. ^{2}H-labeled phenethylamine was not detected by NMR or LCMS.

CHAPTER 4

Synthesis of Enantioenriched Isotopically-labeled α-Amino Acids Using a Chiral Aldehyde Receptor in Conjunction with Isotopically Labeled CO_2

4.1 Introduction

Organocatalysis is a useful strategy for the formation C–C and C–X bonds. The 2021 Nobel prize in chemistry was awarded to B. List and D.W.C. MacMillan for the development of enamine, iminium, and carbonyl catalysis.[181-184] Enamine catalysis is the process which uses amines to activate carbonyl compounds. The mechanism of enamine catalysis involves condensation of a carbonyl compound (**4-a**) with an amine catalyst (**4-b**) to get an imine (**4-c**), which can be converted into an enamine (**4-d**). The reaction of this activated enamine (**4-d**) with an electrophile can proceed to give a substituted carbonyl (**4-f**). On the other hand, carbonyl catalysis uses a carbonyl compound to activate primary amines (**4-b**) through the formation of imines (**4-c**) that can be deprotonated to generate an *α*-amino carbanion (**4-g**). Trapping of the carbanion with an electrophile yields *α*-functionalized amine product (**4-i**) upon hydrolysis of the imine intermediate (**4-h**) (**Fig. 4–1**). In general, carbonyl catalysis enhances the reactivity of the amines *α*-position without protection and deprotection manipulations.[185-186]

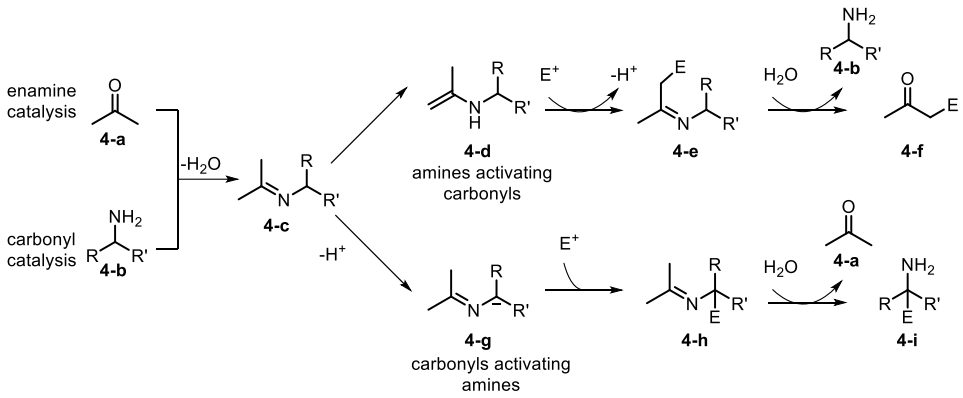


Fig. 4–1. Enamine catalysis vs carbonyl catalysis.

Chiral aldehyde catalysis has been used in organic chemical transformations to mimic biological carbonyl catalysis, which is exemplified by reactions of pyridoxal-dependent enzymes.[186-193] The main chiral aldehyde catalysts that have been used in the asymmetric synthesis of α-amino acids and their derivatives are chiral pyridoxal-dependent catalysts[194-196] and chiral binaphthol (BINOL) aldehyde catalysts (**Fig. 4–2**).[197]

Fig. 4–2. Pyridoxal-dependent catalysis and chiral binaphthol (BINOL) aldehyde catalysis**.**

An example of using these catalysts was in 2014, when Guo et al. reported the use of a chiral BINOL aldehyde catalyst in asymmetric α-alkylation of *N*-unprotected amino esters via imine activation (**Fig. 4–3**).[198] The proposed mechanism for this transformation includes the condensation of chiral binaphthol (BINOL) and dimethyl 2-aminomalonate to form azomethine ylide dipole (**I**), then the hydrogen of 2′-OH of (**I**) complexes with the nitrogen atom of the vinylogous imino intermediate and forms the transition state (**TS I**). The azomethine ylide dipole (**I**) attacks the active intermediate at the si-face and produces the imine (**II**). Imine (**II**) produces the product and regenerates the catalyst by hydrolysis, as shown in **Fig. 4–3**.[198]

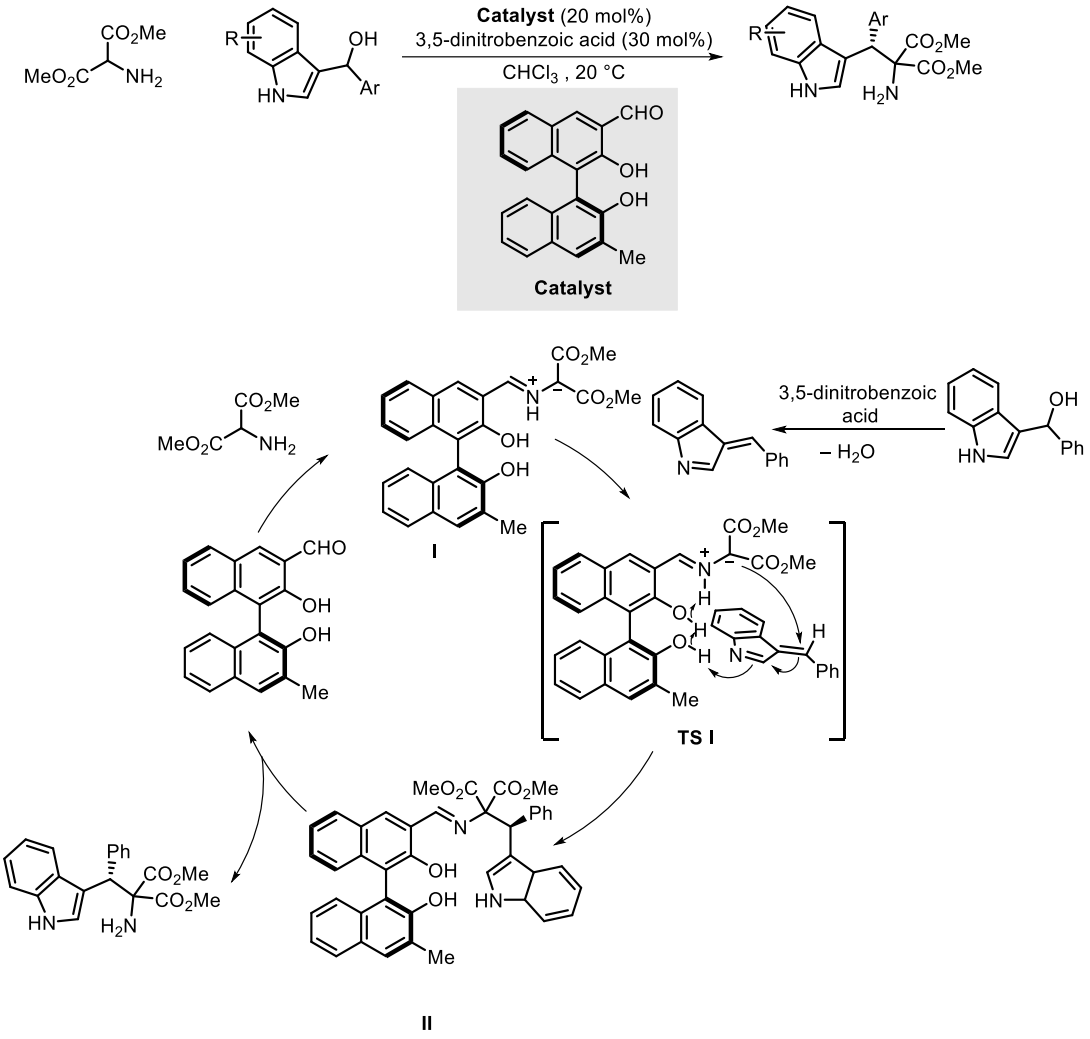


Fig. 4–3. Asymmetric functionalization of the α-amino ester by chiral binaphthol (BINOL) aldehyde catalysis.

In 2018, Zhao et al. designed *N*-quaternized pyridoxals as ideal enzyme mimics that likely were enabled by enzyme-like cooperative bifunctional activation of the substrates (**Fig. 4–4a**) and applied them to asymmetric Mannich reactions of *tert*-butyl glycinate with aryl N-diphenylphosphinyl imines (**Fig. 4–4b**).[199] They proposed that this catalyst can activate both substrates and, similar to an enzyme, it can orient the addition by bringing the two reactants together with a specific spatial arrangement. The proposed catalytic mechanism for the reaction involves condensation of the chiral pyridoxal catalyst with *tert*-butyl glycinate to form an aldimine intermediate. Deprotonation of the α–C–H aldimines generates the active carbanion, which then

undergoes asymmetric addition to the imine moiety, and subsequent hydrolysis of the imine forms the α,β-diamino acid ester and regenerate the catalyst, completing a catalytic cycle, as shown in **Fig. 4–4c**.[199]

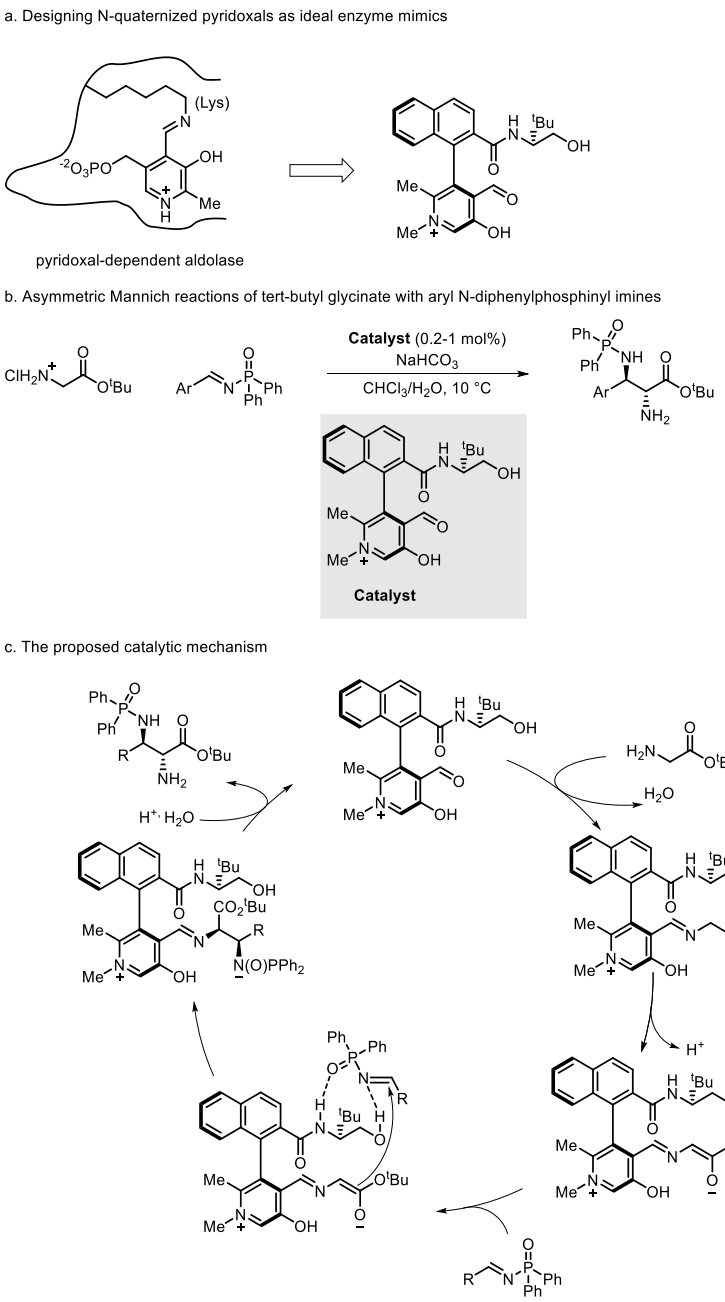
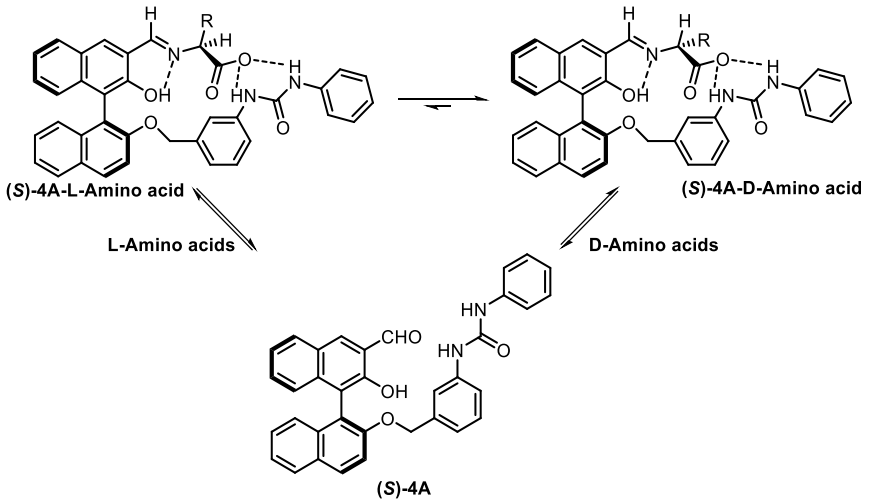


Fig. 4–4. Asymmetric functionalization of the α-amino ester by chiral pyridoxal–dependent catalysis.

Over the past decade, there has been an interest in preparing stereoselective receptors for α-amino acids[200] since they are important building blocks in

chirotechnology.[201] The main challenge in this area has been developing stereoselective receptors for α-amino acids, based on simple organic molecules. Kim et al. have reported the use of chiral binol aldehyde receptors for the inversion of the configuration of 1,2-aminoalcohols[202-204] and L to D α-amino acid inversion through epimerization of a Schiff base intermediate.[205-208] One of the interesting chiral binol aldehyde receptors that they have designed is a urea-based binol aldehyde receptor, which can be used for the inversion of L-amino acids to D-amino acids via Schiff base intermediate epimerization (**Fig. 4–5**).



α-amino acids	D/L ratio	α-amino acids	D/L ratio
threonine	95/5	methionine	92/8
glutamine	94/6	glutamic acid	92/8
histidine	93/7	serine	92/8
arginine	93/7	leucine	90/10
asparagine	93/7	tryptophan	89/11
tyrosine	92/8	alanine	88/12
phenylalanine	92/8		

Fig. 4–5. Epimerization of α-amino acids via urea-based binol aldehyde receptor and the ratios of [**(*S*)-4A-D-aa**] to [**(*S*)-4A-L-aa**] at equilibrium.

The urea-based binol aldehyde, (*S*)-2-hydroxy-2'-(3-phenyluryl-benzyl)-1,1'-binaphthyl-3-carboxaldehyde ((***S*)-4A**) binds with α-amino acids stereoselectively by

reversible formation of an imine bond with internal resonance-assisted hydrogen bonding (RAHB).[209] This urea-based binol aldehyde is capable of converting most proteinogenic L-amino acids to D-amino acids, with an equilibrium ratio (D:L) for the diastereomers of the imine condensation product ranging from (88:12) for alanine to (95:5) for threonine, as shown in **Fig. 4–5**.

In general, the literature methods to prepare isotopically-labeled enantioenriched α-amino acids depend on the insertion of labeled carbon via classical methods, followed by resolution. The resolution methods can be carried out by an enzymatic approach after the insertion of labeled carbon. The enzymatic approach includes enzymatic kinetic resolution by an acylase enzyme, as shown in **Fig. 4–6a**. The acylase enzyme catalyzes the hydrolysis of L-*N*-acetyl methionine, which allowed [C_1-^{13}C]methionine to be obtained in 99.9:0.1 *er.*[147-149] Kinetic resolution by acylase enzyme is specific for α-amino acids that contain hydrophobic groups, such as methionine and leucine.

The other enzymatic approach utilizes phenylalanine lyase (PAL) enzyme for stereoselective C–N bond formation, as shown in **Fig. 4–6b**.[152-153] This enzyme catalyzes the addition of ammonia to ®-cinnamic acid, resulting in formation of [C_1-^{13}C]phenylalanine to be obtained in 99.9:0.1 *er*.[152] The lyase enzyme class is specific for aromatic α-amino acids, such as phenylalanine, tyrosine, tryptophan, and their derivatives.[152]

The resolution of racemic amino acids also can be carried out using chiral auxiliaries for α-alkylation, as shown in **Fig. 4–6c**.[154] This method is specific for α-amino acids that contain hydrophobic side-chains, such as leucine, valine, and isoleucine. This method involves the use of a chiral auxiliary derived from Evans' oxazolidinone imides. Reaction of this chiral auxiliary with *n*-butyllithium, followed by butyryl chloride gives the corresponding oxazolidinone in quantitative yield. This oxazolidinone is deprotonated by sodium bis(trimethylsilyl)amide (NaHMDS) to give the124orresponddding sodium enolate. Alkylation of this sodium enolate with isotopically-labeled methyl iodide [^{13}C]CH_3I installs the isotopic label. Cleavage of the resultant chiral auxiliary with lithium hydroxide and hydrogen peroxide gives

(*S*)-2-methylbutanoic acid. A one-carbon homologation of the resultant carboxylic acid gives the required α-keto ester. This is followed by biotransformations, using leucine dehydrogenase (LeuDH) enzyme, reduced nicotinamide adenine dinucleotide (NADH), and formate dehydrogenase (FDH). This enzymatic transformation promotes hydrolysis of the ester and reductive amination of the resultant α-keto acid to furnish [C_1-^{13}C]isoleucine with 99.9:0.1 *er* , as shown in **Fig. 4–6c**.[154]

Another method to prepare isotopically-labeled enantioenriched α-amino acids after the insertion of labeled carbon via classical methods is the enantioselective hydrogenation of enamines with a chiral rhodium (I) catalyst (**Fig. 4–6d**). This method is designed to prepare triply isotopically-labeled serine, cystine, and alanine in a multistep synthetic pathway. [155]

All these methodologies to access enantiomerically pure isotopically labeled α-amino acids are limited by a narrow substrate scope, multistep procedures, and late-stage resolution.

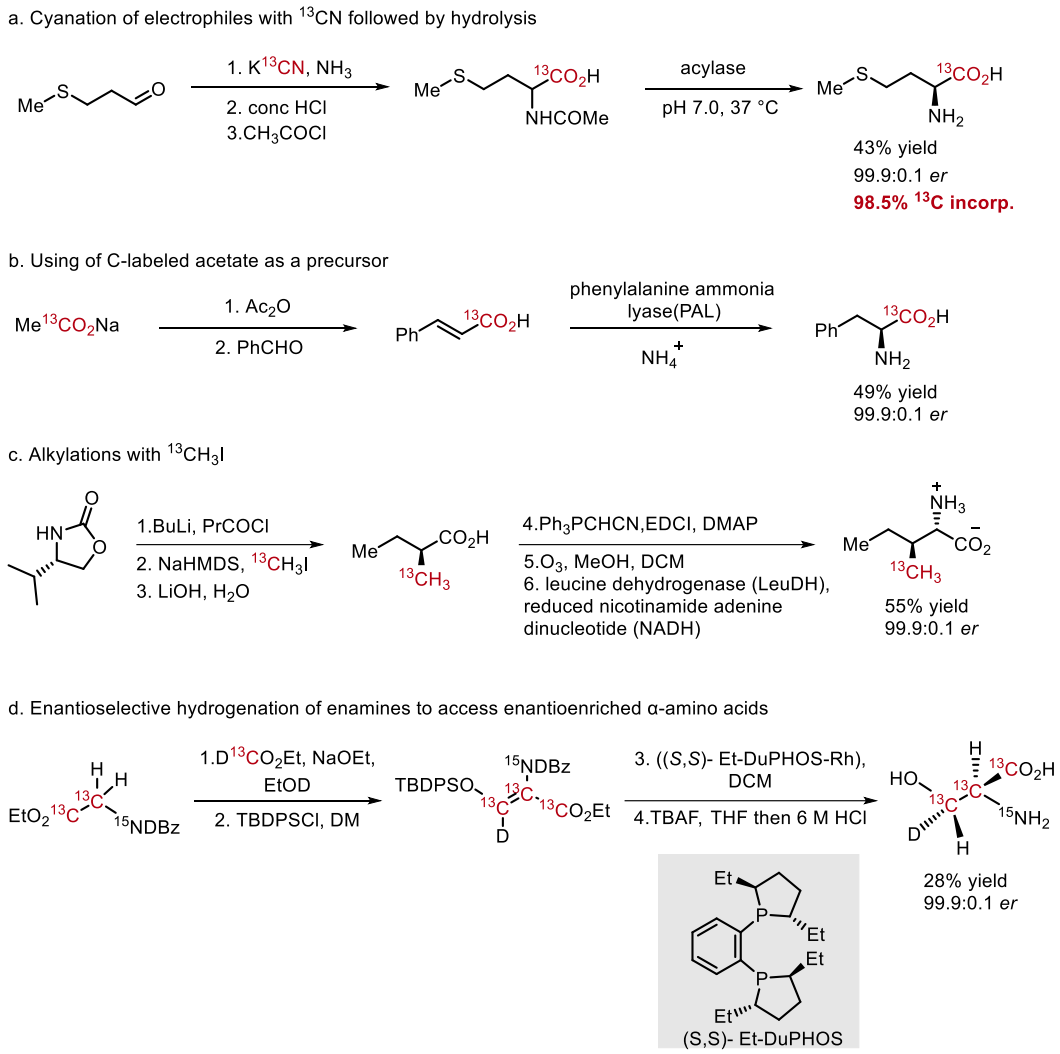


Fig. 4–6. Literature methods to prepare isotopically-labeled enantioenriched α-amino acids.

The preparation of isotopically-labeled enantioenriched α-amino acids remains difficult and time-consuming, with established methods involving label incorporation at an early stage of synthesis and resolution at a later stage of synthesis in multi-step synthesis pathways. Motivated by this methodological gap and the need for isotopically-labeled enantioenriched α-amino acids for PET imaging and clinical studies, Section 4.2 describes the development of the synthesis of enantioenriched isotopically-labeled α-amino acids, using chiral aldehyde receptor in conjunction with isotopically-labeled CO_2.

4.2 Developement of Chiral Aldehyde Receptor Catalyzed Carboxylate Exchange in α-Amino Acids with Isotopically-labeled CO_2

4.2.1 Discovery and Optimization of the Methodology

The urea-based binol aldehyde receptors are known catalysts for the inversion of L-amino acids to D-amino acids via shift base intermediate epimerization.[204-207] Also, we know that an achiral aryl aldehyde catalyst can catalyze carboxylate exchange in α-amino acids (Chapter 3);[210] therefore, the big question that arises is about the possibility of using a chiral aldehyde to induce carboxylate exchange and resolution in a single synthetic operation. The enantioenriched isotopically-labeled α-amino acids were accessed after the isotopic labeling of α-amino acids by achiral aryl aldehydes in Chapter 3 via literature methods, including Ni catalyzed DKR,[171] enzymatic KR,[169-170] and chromatographic resolution, as shown in **Fig. 4–7**.[206] Although the isotopic labeling of α-amino acids by achiral aryl aldehydes[210] and then resolution methods led to enantioenriched isotopically labeled α-amino acids in three steps, they gave a moderate yield of the final product, and they worked only with α-amino acids that contain hydrophobic groups, like methionine and phenylalanine. Therefore, the development of a direct resolution/carboxylate exchange reaction for α-amino acid labeling via a single step and for all proteinogenic amino acids and non-natural amino acids would be the conceptually ideal technology to access enantioenriched isotopically-labeled α-amino acids.

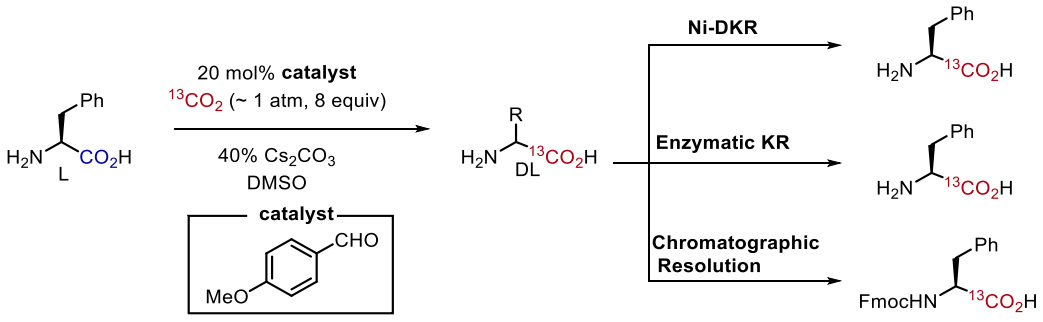
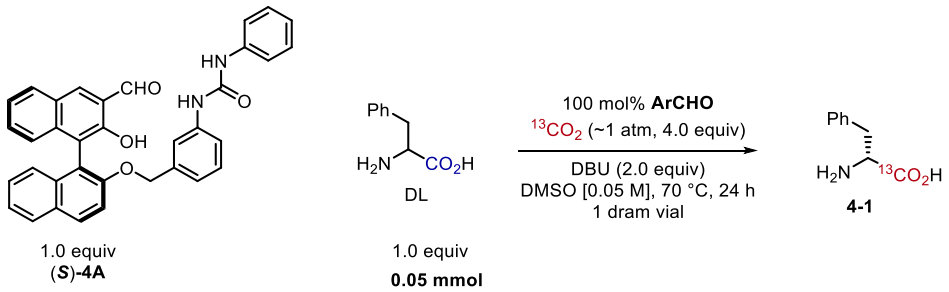


Fig. 4–7. Carboxylate exchange/deracemization strategies.

The urea-based binol aldehyde ((***S***)-**4A**) was prepared according to a literature procedure.[209] Initially, upon using conditions from Chapter 3,[210] a catalytic amount of

urea-based binol aldehyde ((***S***)-**4A**) gave low *er* (52:48) and low incorporation, as shown in **Fig. 4–8**. This led to considerations about the chiral aldehyde catalyst ((***S***)-**4A**) as a receptor and the need for using it stoichiometrically with α-amino acids. After a wide range of screening the chiral aldehyde ((***S***)-**4A**) loading, bases, and solvents, we found that in the presence of 100 mol% urea-based binol aldehyde ((***S***)-**4A**) and 200 mol% DBU in DMSO at 70 ºC, C1-resolution/carboxylate exchange in (±)-phenylalanine to generate (D)-[C_1-^{13}C]phenylalanine is observed with 66% ^{13}C incorporation, 66% yield, and 90:10 *er* when ~4 equivalents of [^{13}C]CO_2 are supplied (This provides ~4 equivalents (~0.2 mmol) of $^{13}CO_2$, which would result in an equilibrium exchange incorporation of ~80%). **Fig. 4–8** provides an overview of experimental parameters important to observing productive α-amino acid resolution/carboxylate exchange with externally supplied $^{13}CO_2$. Using 50 mol% urea-based binol aldehyde ((***S***)-**4A**) led to low *er* (64:36) and lower incorporation. Using Cs_2CO_3 as a base instead of DBU gave similar *er* (90:10) and lower incorporation. Other organic bases, such as DIPEA and TBD gave lower *er*. Performing the reaction at 60 ºC resulted in similar *er* to the standard condition but lower incorporation, while doing the reaction at 80 ºC, gave higher incorporation than the standard conditions, and the *er* was lower. While the use of the chiral aldehyde receptor ((***S***)-**4A**) in a stoichiometric amount with α-amino acid under standard reaction conditions is needed, it is technically a catalyst because the bulk of it can be recovered unchanged after aqueous workup. Under the standard conditions ((***S***)-**4A**) can be recovered after the reaction is completed in 70% isolated yield, as shown in **Fig. 4–9**.



variation to standard conditions	^{13}C inc. (%)	y (%)	er (D:L)
no change	**66**	66	90:10
20% of Cat 1 instead of 100%	**18**	94	52:48
50% of Cat 1 instead of 100%	**48**	74	64:36
1.2 equiv of Cat 1 instead of 1.0 equiv	**64**	66	89:11
Cs_2CO_3 instead of DBU	**36**	74	90:10
DIPEA instead of DBU	**24**	64	76:24
TBD instead of DBU	**71**	70	80:20
60 °C instead of 70 °C	**46**	64	91:9
80 °C instead of 70 °C	**78**	54	86:14
1.0 equiv of DBU instead of 2.0 equiv	**19**	69	90:10
3.0 equiv of DBU instead of 2.0 equiv	**70**	56	90:10

Fig. 4–8. Overview of impact of reaction parameters on the resolution/carboxylate exchange of α-amino acids.

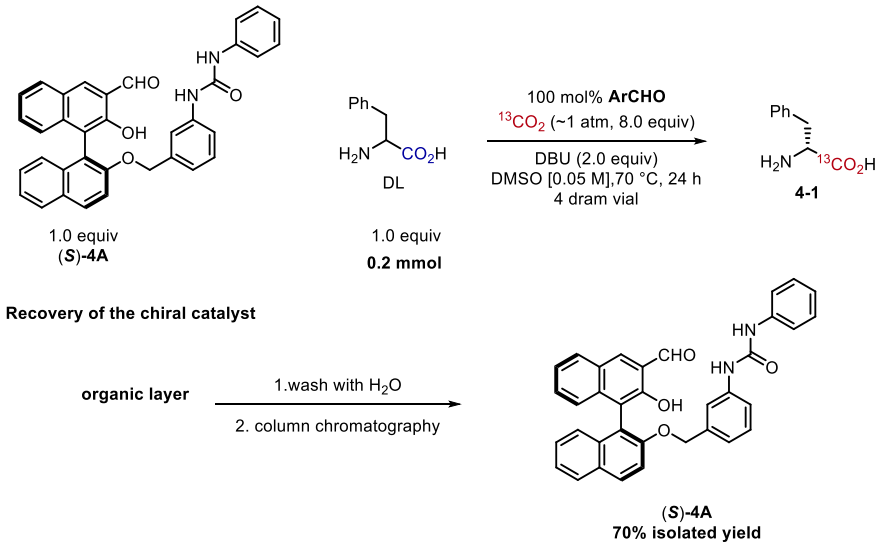


Fig. 4–9. Recovery of chiral aldehyde catalyst.

4.2.2 Scope of the Methodology

The chiral aldehyde ((***S***)-**4A**) mediates resolution/carboxylate exchange in a diverse range of unprotected α-amino acids, including most proteinogenic substrates and non-natural variants containing potentially reactive functional groups (**Fig. 4–10**). Aliphatic and aromatic α-amino acids can be labelled in 41–88% ^{13}C-incorporation with >50% yield and up to 90:10 *er,* including phenylalanine, methionine, alanine, leucine, tyrosine, and tryptophan (**4**–**1** to **4**–**6**). Basic side-chain groups generally were well-tolerated to give 60–87% incorporation of label with good enantiomeric ratio (**4**–**7**, **4**–**8**). Unsuccessful examples of this methodology include isoleucine and threonine (**4-9**, **4-10**). Both isoleucine and threonine gave low incorporation and recovery; in addition to that, isoleucine does not work under resolution conditions by using chiral aldehyde ((***S***)-**4A**).[209]

As shown in **Fig. 4–10**, the enantioenrichment carboxylate exchange process is not impeded by various functional groups, as seen in phenylalanine derivatives containing fluoride, bromide, and iodide groups (**4**–**11**, **4**–**12**, **4**–**13**), or azide (**4-14**), and nitro (**4-15**) units.

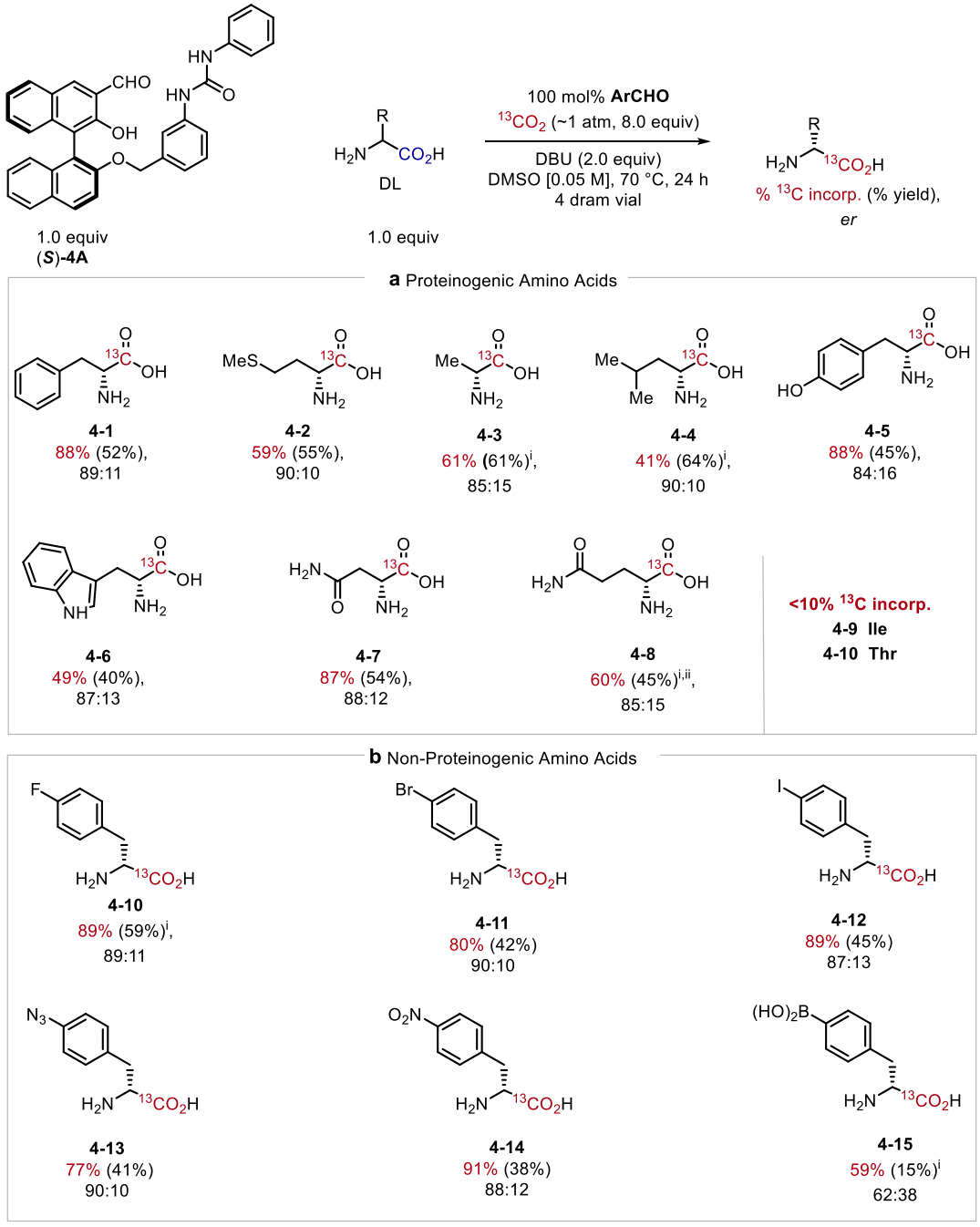


Fig. 4–10. A. Chiral aldehyde-catalyzed carboxylate exchange of proteinogenic α-amino acids with [^{13}C]CO_2. B. Scope with non-proteinogenic α-amino acids. [i] Yield determined by ^{1}H NMR spectroscopy. [ii] Reaction carried out at 60 °C.

4.2.3 Enantioenriched Carboxylate Exchange/D-Amino Acid Oxidase (DAAO) to Access Isotopically-labeled Enantiomerically Pure α-Amino Acids

Under the standard reaction conditions, the urea-based binol aldehyde (®-**4A**), mediates the C1-enantioselective carboxylate exchange of phenylalanine and methionine, generating [C_1-^{13}C]phenylalanine, [C_1-^{13}C]methionine with 90:10 *er.* For potential applications where higher enantiopurity is needed, upgrading the enantiomeric ratio can be achieved by using an oxidase enzyme. Oxidases are a major biotechnological interest because they can catalyze the selective oxidation of organic compounds under mild reaction conditions by using molecular oxygen as an oxidizing agent.[211] D-Amino acid oxidases (DAAO) are interesting examples of this group of enzymes. They can catalyze the oxidative deamination of a wide spectrum of D-amino acids to yield their corresponding α-keto acid.[211] Performing the standard reaction for (±)-phenylalanine and (±)-methionine, using chiral aldehyde (®-**4A**) generates the corresponding isotopically labelled amino acid with up to 90:10 *er.* The upgrade of this enantiomeric ratio was achieved by using D-amino acid oxidases (DAAO) and catalase in a phosphate buffer to oxidize the minor D-enantiomer, as shown in **Fig. 4–11**, to generate the corresponding naturally occurring isotopically-labeled α-amino acid in 99.9:0.1 *er,* with 48% isolated yield over two steps for phenylalanine and 99.5:0.5 *er* with 55% isolated yield over two steps for methionine.

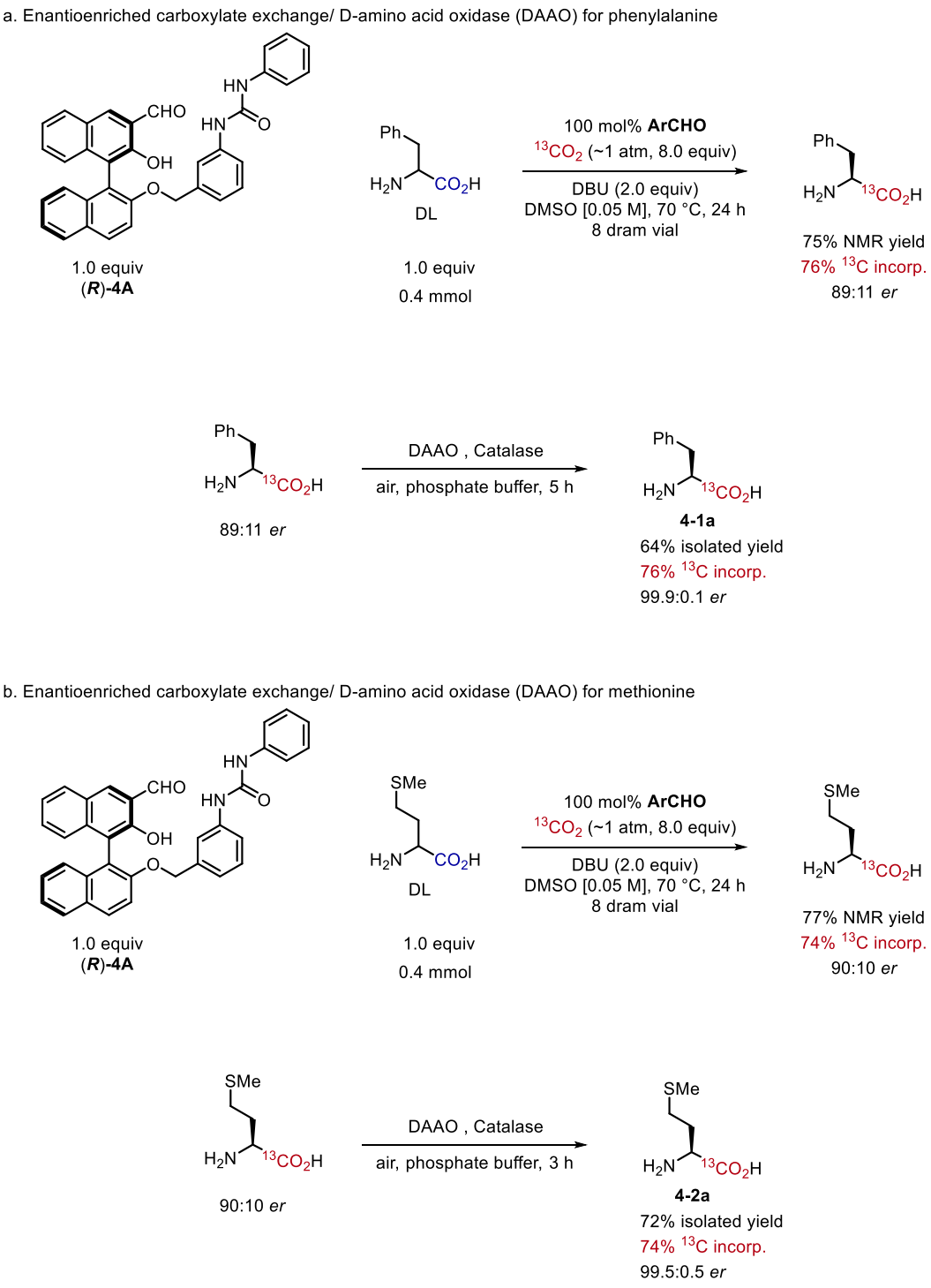


Fig. 4–11. Enantioenriched carboxylate exchange/D-amino acid oxidase (DAAO) to access isotopically-labeled enantiomerically pure α-amino acids. a. for (±)-phenylalanine., b. for (±)-methionine.

4.2.4 Development of Fast Exchange Conditions to Enable Direct Radiolabeling of Enantioenriched α-Amino Acids Using [^{11}C]CO_2

Modification of the reaction conditions provides some evidence that fast exchange conditions to enable direct radiolabeling of α-amino acids, using [^{11}C]CO_2, could be possible. To promote faster reactions, pre-formed imine carboxylates were generated quantitatively by condensation of α-amino acids with chiral aldehyde ((***S***)-**4A**) in basic MeOH[136] and examined as reagents for carboxylate exchange. The best conditions were by running the exchange at 120 °C for 10 min to get 44% ^{13}C incorporation with 77:23 *er*, followed by a cool-down to 70 °C for another 10 min to get the equilibrium ratio of D to L (90:10), 47% incorporation, and 62% NMR yield (**Fig. 4–12**).

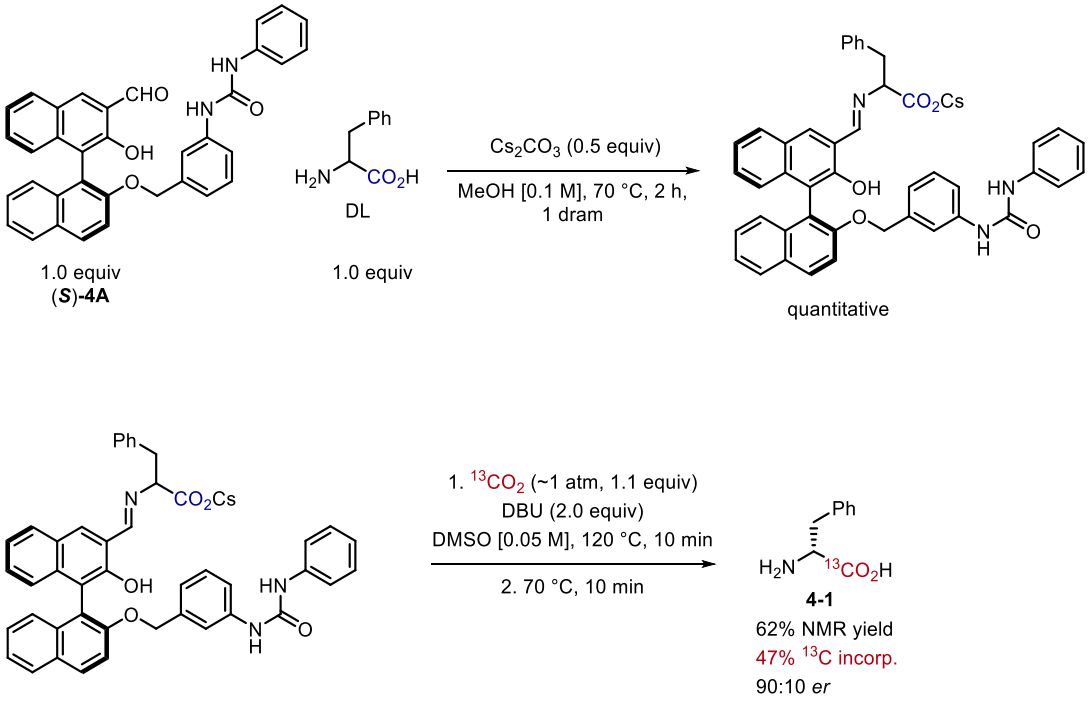


Fig. 4–12. Development of fast conditions for enantioenriched carboxylate exchange.

4.3 Summary and Conclusions

In summary, a new strategy is reported for the resolution/isotopic labeling of α-amino acids mediated by using a urea-based binol aldehyde receptor with conjunction of [^{13}C]CO_2 via reversible decarboxylation/carboxylation event of an imine carboxylate intermediate. Our developed method provides access to C1-enantioenriched-labeled

products in a direct and operationally trivial manner. We also have shown that after getting C1- enantioenriched labeled α-amino acids, they can be converted readily to enantiomerically-labeled naturally occurring α-amino acids via D-amino acid oxidase and catalase. Given the widespread use of enantiomerically-labeled α-amino acids in discovery science, drug development, and medical imaging, we expect this finding to have immediate application.

4.4 Procedures and Characterization

General Considerations

Unless noted, all reactions were conducted under an inert atmosphere, employing standard Schlenk techniques or by the use of an N_2-filled glovebox. All glassware was oven-dried prior to use. Flash chromatography was performed, as described by Still and co-workers[128] (SiliaFlash P60, 40-63μm, 60A silica gel, Silicycle) or by automated flash chromatography (KP-C18-HS 60g). Analytical thin-layer chromatography was performed, using glass plates pre-coated with silica (SiliaPlate G TLC - Glass-Backed, 250μm, Silicycle). TLC plates were visualized by staining with aqueous acidic ninhydrin and/or by UV light. NMR spectra (^1H, ^{13}C) were obtained on an Agilent VNMRS 700 MHz, Varian VNMRS 600 MHz, Varian VNMRS 500 MHz, or Varian VNMRS 400 MHz spectrometer. The chemical shifts are given as parts per million (ppm) and were referenced to the residual solvent signal ($CDCl_3$: δH = 7.26 ppm, δC = 77.06 ppm) (DMSO-d_6: δH = 2.49 ppm, δC = 39.50 ppm) (Acetone-d_6: δH = 2.04 ppm, δC = 29.80 ppm) (MeOH-d_4: δH = 3.30 ppm, δC = 49.00 ppm). HRMS analyses of ^{13}C labelled compounds were performed on an Agilent Technologies 6220 oaTOF instrument (ESI, APPI, APCI) in positive or negative ionization mode. Crude LC-MS analyses of ^{13}C labelled compounds were performed on an Agilent Technologies 1260 system with G6130B MSD single quadrupole MS (ESI) in positive or negative ionization mode, using a Phenomenex Kinetex C8 column (2.1 × 50 mm, 1.7 μm particle size) or a Phenomenex Luna Omega C18 Polar column (2.1 × 50 mm, 1.6 μm particle size) in reverse-phase. Chiral HPLC analysis was accomplished on an Agilent Technologies 1100 system with an Astec Chirobiotic-T column (4.6 × 100 mm, 5 μm particle size) under reverse-phase conditions. Preparative HPLC was accomplished,

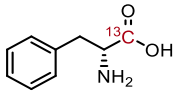
using an Agilent 1260 Infinity system under reverse-phase conditions (Agilent Prep-C18 column, 21.2 × 150 mm, 10 μm particle size). Unless otherwise noted, quantitative ^{1}H NMR yields were determined from crude reaction mixtures, using 3-(trimethylsilyl)-1-propanesulfonic acid (DSS) as an internal standard. Unless otherwise noted, all reagents were obtained from commercial vendors (Sigma-Aldrich, Combi-Blocks, Alfa Aesar, Acros, and TCI) and used as supplied. [^{13}C]CO_2 (99.0 atom % ^{13}C) and anhydrous DMSO were purchased from Sigma-Aldrich.

General Procedures and Characterization

General Procedure A for optimization: In an atmosphere-controlled glovebox, DL-^{12}C-phenylalanine (0.05 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (26.9 mg, 0.05 mmol, 1.00 equiv.), DBU (14.9 μL, 0.2 mmol, 2.0 equiv.), and sodium trimethylsilylpropanesulfonate internal standard (DSS) were added sequentially to a 1-dram vial charged with a stir bar, followed by the addition of anhydrous DMSO (1.0 mL). The vial was sealed with a PTFE-lined cap and removed from the glovebox. The reaction headspace was evacuated on a Schlenk line (~300 mtorr), using a 25-gauge needle. The vial headspace was carefully refilled with 15 psi [^{13}C]CO_2 through the PTFE-lined cap with a 25-gauge needle until the internal pressure reached ~1 atm (requires 20–60 s, depending on the pressure of the [^{13}C]CO_2 tank). This provides ~4 equivalents (~0.2 mmol) of [^{13}C]CO_2, which would result in an equilibrium exchange incorporation of ~80%. The vial cap was sealed with parafilm and electrical tape, and the reaction was stirred at the corresponding temperature in an aluminum block until completion of the reaction (24 h). To determine the percent recovery of the amino acid, a small aliquot (~5 μL) of the reaction was placed in 0.70 mL DMSO for calibrated ^{1}H NMR analysis, using DSS as the reference signal. A small aliquot of the reaction (~5 μL) was placed in 1.0 mL of 1:1 MeOH:H_2O/0.1% HCO_2H for LC-MS analysis to determine the crude ^{13}C% incorporation of the amino acid. The reaction mixture was quenched at the corresponding temperature with 1 mL of 2 M FA and then diluted with H_2O (5 mL). The aqueous layer was washed with DCM (5 × 5 mL) and was lyophilized to remove the H_2O. The *er*% was obtained through chiral HPLC analysis of the products.

General Procedure B for scope studies: In an atmosphere-controlled glovebox, amino acid (0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.20 mmol, 1.00 equiv.), DBU (59.6 μL, 0.4 mmol, 2.0 equiv.), and sodium trimethylsilylpropanesulfonate internal standard (DSS) were added sequentially to a 4-dram vial charged with a stir bar, followed by the addition of anhydrous DMSO (4.0 mL). The vial was sealed with a PTFE-lined cap and removed from the glovebox. The reaction headspace was evacuated on a Schlenk line (~300 mtorr), using a 25-gauge needle. The vial headspace was carefully refilled with 15 psi [^{13}C]CO_2 through the PTFE-lined cap with a 25-gauge needle until the internal pressure reached ~1 atm (requires 20–60 s, depending on the pressure of the [^{13}C]CO_2 tank). This provides ~8 equivalents (~1.6 mmol) of $^{13}CO_2$, which would result in an equilibrium exchange incorporation of ~85%. The vial cap was sealed with parafilm and electrical tape, and the reaction was stirred at 70 °C in an aluminum block until completion of the reaction (24 h). To determine the percent recovery of the amino acid, a small aliquot (~5 μL) of the reaction was placed in 0.70 mL DMSO for calibrated ^{1}H NMR analysis, using DSS as the reference signal. A small aliquot of the reaction (~5 μL) was placed in 1.0 mL of 1:1 MeOH:H_2O/0.1% HCO_2H for LC-MS analysis to determine the crude ^{13}C% incorporation of the amino acid. The reaction mixture was quenched at 70 °C with 1 mL of 2 M FA and then diluted with H_2O (5 mL). The aqueous layer was washed with DCM (5 × 5 mL) and was lyophilized to remove the H_2O. Then, the crude mixture was dissolved in a minimum a mount of H_2O, loaded to a flash chromatography column (KP-C18-HS 60 g), and purified by reverse phase chromatography (2%-5% MeCN in H_2O). The *er*% was obtained through chiral HPLC analysis of the products. The ^{13}C% incorporation was obtained through high resolution mass spectrometry (HRMS) analysis of the isolated products.

Specific Experimental Details and Product Characterization Data



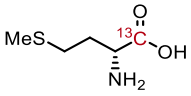
4-1 Prepared according to the general procedure B from DL-^{12}C-phenylalanine (33.0 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 µL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 61%. Isolated in 52% yield, *er:* 88:12, 88% ^{13}C incorporation (HRMS) as an off-white solid after purification by preparative-HPLC (Agilent Prep-C18 column, 2.5% MeOH in H_2O, 25 mL/min).

^{1}H NMR (D_2O, 500 MHz) δ 7.40–7.38 (m, 2H), 7.35–7.32 (m, 1H), 7.30–7.29 (m, 2H), 3.93 (m, 1H), 3.23 (brs, 1H), 3.09 (brs, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 175.3, 136.2, 130.3, 130.0, 128.6, 56.9 (d, J = 56.2 Hz), 37.5;

HRMS (ESI): calcd. for $C_8[^{13}C]H_{10}NO_2$ $[M-H]^-$: 165.0751. Found 165.0751;

Chiral HPLC: 89:11 *er*. Determined on Astec Chirobiotic-T column (60% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 9.6 min (minor), t_r = 10.6 min (major).



4-2 Prepared according to the general procedure B from DL-^{12}C-methionine (29.8 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 µL 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 74%. Isolated in 55% yield, *er:* 90:10, 59% ^{13}C incorporation (HRMS) as an off-white solid after purification by reverse phase column chromatography (5% MeCN in H_2O).

^{1}H NMR (D_2O, 500 MHz) δ 3.91–3.94 (m, 1H), 2.71 (t, J = 7.4 Hz, 2H), 2.25–2.30 (m, 1H), 2.16–2.20 (m, 4H);

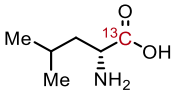
^{13}C NMR (D_2O, 125 MHz) δ 175.2, 54.7 (d, J = 53.3 Hz), 30.6, 29.8, 14.9;

HRMS (ESI): calcd. for $C_4[^{13}C]H_{10}NO_2S$ $[M-H]^-$: 149.0471. Found 149.0470;

Chiral HPLC: 90:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.3 min (minor), t_r = 13.2 min (major).

4-3 Prepared according to the general procedure B from DL-^{12}C-alanine (17.8 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)-**4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 µL 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 61%. *er:* 85:15, 61% ^{13}C incorporation (LCMS).

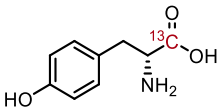
Chiral HPLC: 85:15 *er*. Determined on Astec Chirobiotic-T column (60% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.3 min (minor), t_r = 12.1 min (major).



4-4 Prepared according to the general procedure B from DL-^{12}C-leucine (26.2 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)-**4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 µL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 64%. *er:* 90:10, 41% ^{13}C incorporation (HRMS).

HRMS (ESI): calcd. for $C_5[^{13}C]H_{12}NO_2$ $[M-H]^-$: 131.0902. Found 131.0908;

Chiral HPLC: 90:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.3 mL/min), t_r = 17.9 min (minor), t_r = 21.8 min (major).



4-5 Prepared according to the general procedure B from DL-^{12}C-tyrosine (36.2 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)-**4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 µL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 66%. Isolated in 45% yield, *er:*

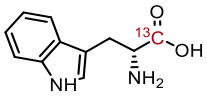
84:16, 88% ^{13}C incorporation (HRMS) as an off-white solid after purification by reverse phase column chromatography (2% MeCN in H_2O).

^{1}H NMR (D_2O, 500 MHz) δ 7.08–7.10 (d, *J* = 8.2 Hz, 2H), 6.76–6.78 (d, *J* = 8.2 Hz, 2H), 3.62 (brs, 1H), 2.99 (brs, 1H), 2.84 (brs, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 180.0, 158.4, 131.7, 127.8, 117.4, 58.1 (d, *J* = 52.9 Hz), 38.9;

HRMS (ESI): calcd. for $C_8[^{13}C]H_{10}NO_3$ $[M-H]^-$: 181.0700. Found 181.0671;

Chiral HPLC: 84:16 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 9.6 min (minor), t_r = 10.8 min (major).



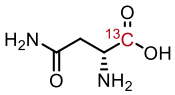
4-6 Prepared according to the general procedure B from DL-^{12}C-tryptophan (40.8 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 μL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 56%. Isolated in 40% yield, *er:* 87:14, 49% ^{13}C incorporation (HRMS) as an off-white solid after purification by reverse phase column chromatography (5% MeCN in H_2O).

^{1}H NMR (D_2O, 500 MHz) δ 7.80 (d, *J* = 8.4 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.74 (br s, 1H), 7.33–7.36 (m, 1H), 7.25–7.28 (m, 1H), 4.11–4.13 (m, 1H), 3.5–3.57 (m, 1H), 3.37 (dd, *J* = 15.5, 8.2 Hz, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 175.4, 137.3, 127.6, 125.9, 123.1, 120.4, 119.4, 112.9, 108.4, 56.1 (d, *J* = 53.4 Hz), 27.4;

HRMS (ESI): calcd. for $C_{10}[^{13}C]H_{11}N_2O_2$ $[M-H]^-$: 204.0860. Found 204.0861;

Chiral HPLC: 87:13 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.6 min (minor), t_r = 11.9 min (major).



4-7 Prepared according to the general procedure B from DL-^{12}C-asparagine (26.4 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00

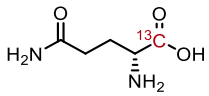
equiv.), and DBU (59.6 μL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 61%. Isolated in 54% yield, *er:* 88:12, 87% ^{13}C incorporation (HRMS) as an off-white solid after purification by reverse phase column chromatography (2% MeCN in H_2O).

^{1}H NMR (D_2O, 500 MHz) δ 4.04–4.08 (m, 1H), 3.01 (dt, *J* = 16.9, 4.2 Hz, 1H), 2.91 (dd, *J* = 16.9, 7.7 Hz, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 174.3, 52.4 (d, *J* = 53.4 Hz), 35.6;

HRMS (ESI): calcd. for $C_3[^{13}C]H_7NO_3$ $[M-H]^-$: 132.0496. Found 132.0496;

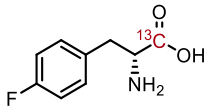
Chiral HPLC: 87:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 11.5 min (minor), t_r = 13.8 min (major).



4-8 Prepared according to the general procedure B from DL-^{12}C-glutamine (29.2 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 μL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction was stirred at 60 °C for 24 h. The reaction mixture was quenched at 60 °C with 1 mL of 1 M HCl then diluted with H_2O (5 mL). The aqueous layer was washed with DCM (5 × 5 mL) and was lyophilized to remove the H_2O. ^{1}H NMR yield: 45%, *er:* 84:16, 60% ^{13}C incorporation (HRMS).

HRMS (ESI): calcd. for $C_4[^{13}C]H_9N_2O_3$ $[M-H]^-$: 146.0652. Found 146.0654;

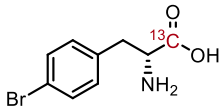
Chiral HPLC: 84:16 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 11.5 min (minor), t_r = 13.8 min (major).



4-11 Prepared according to the general procedure B from DL-^{12}C-4-fluorophenylalanine (36.6 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 μL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 59%, *er:* 89:11, 89% ^{13}C incorporation (HRMS).

HRMS (ESI): calcd. for $C_8[^{13}C]H_9FNO_2$ $[M-H]^-$: 183.0656. Found 183.0655;

Chiral HPLC: 89:11 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 9.3 min (minor), t_r = 10.3 min (major).



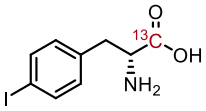
4-12 Prepared according to the general procedure B from DL-^{12}C-4-bromophenylalanine (48.8 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)-**4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 µL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 51%. Isolated in 42% yield, *er:* 90:10, 80% ^{13}C incorporation (HRMS) as an off-white solid after purification by preparative-HPLC (Agilent Prep-C18 column, 2.5% MeCN in H_2O, 15 mL/min).

^{1}H NMR (D_2O, 500 MHz) δ 7.59 (d, *J* = 7.4 Hz, 2H), 7.25 (d, *J* = 7.4 Hz, 2H), 3.65 (brs, 1H), 3.1 (brs, 1H), 2.97 (brs, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 181.3, 137.7, 132.4, 132.2, 120.9, 58.1 (d, *J* = 53.5 Hz), 40.2;

HRMS (ESI): calcd. for $C_8[^{13}C]H_9NO_2[^{79}Br]$ $[M-H]^-$: 242.9856. Found 242.9851;

Chiral HPLC: 90:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.6 min (minor), t_r = 11.7 min (major).



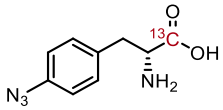
4-13 Prepared according to the general procedure B from DL-^{12}C-4-iodophenylalanine (58.2 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 µL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 48%. Isolated in 45% yield, *er:* 87:13, 89% ^{13}C incorporation (HRMS) as an off-white solid after purification by reverse phase column chromatography (2% MeCN in H_2O).

^{1}H NMR (D_2O, 500 MHz) δ 7.84 (d, *J* = 7.8 Hz, 2H), 7.16 (d, *J* = 7.8 Hz, 2H), 3.99–4.02 (m, 1H), 3.25–3.29 (m, 1H), 3.11–3.15 (m, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 175.1, 138.9, 135.9, 132.3, 93.4, 56.8 (d, *J* = 54.3 Hz), 37.1;

HRMS (ESI): calcd. for $C_8[^{13}C]H_9NO_2I$ $[M-H]^-$: 290.9717. Found 290.9714;

Chiral HPLC: 87:13 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.9 min (minor), t_r = 12.1 min (major).



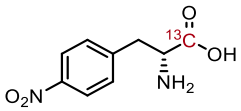
4-14 Prepared according to the general procedure B from L-^{12}C-4-azidophenylalanine (41.2 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 μL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 59%. Isolated in 41% yield, *er:* 90:10, 77% ^{13}C incorporation (HRMS) as an off-white solid after purification by preparative-HPLC (Agilent Prep-C18 column, 2.5% MeCN in H_2O, 15 mL/min).

^{1}H NMR (D_2O, 500 MHz) δ 7.34 (d, *J* = 8.2 Hz, 2H), 7.13 (d, *J* = 8.2 Hz, 2H), 3.68 (brs, 1H), 2.99–3.11 (m, 1H), 2.9–2.95 (m, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 182.7, 139.1, 135.7, 131.7, 119.9, 58.5 (d, *J* = 53.5 Hz), 40.8;

HRMS (ESI): calcd. for $C_8[^{13}C]H_9N_4O_2$ $[M-H]^-$: 206.0765. Found 206.0763;

Chiral HPLC: 90:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.7 min (minor), t_r = 12.2 min (major).



4-15 Prepared according to the general procedure B from DL-^{12}C-4-nitrophenylalanine (0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 μL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0

mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 45%. Isolated in 38% yield, *er:* 88:12, 91% ^{13}C incorporation (HRMS) as an off-white solid after purification by preparative-HPLC (Agilent Prep-C18 column, 2.5% MeCN in H_2O, 15 mL/min).

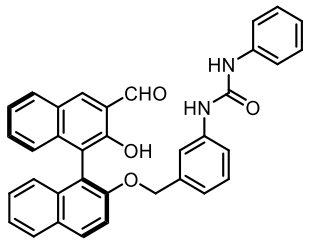
^{1}H NMR (D_2O, 500 MHz) δ 8.31 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 4.05–4.08 (m, 1H), 3.41–3.44 (m, 1H), 3.29–3.34 (m, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 174.7, 148.01,144.6, 131.4, 125.01, 56.5 (d, *J* = 52.6 Hz), 37.5;

HRMS (ESI): calcd. for $C_8[^{13}C]H_9N_2O_4$ $[M-H]^-$: 210.0601. Found 210.0600;

Chiral HPLC: 88:12 *er*. Determined on Astec Chirobiotic-T column (60% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.2 min (minor), t_r = 11.1 min (major).

Recovery of Chiral Aldehyde Catalyst



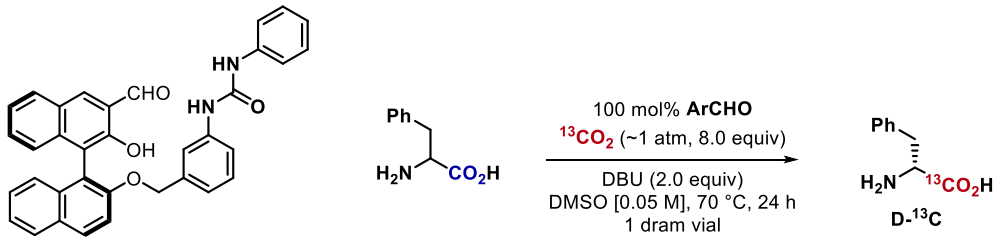
Prepared according to the general procedure B form DL-^{12}C-phenylalanine (33.0 mg, 0.20 mmol, 1.0 equiv.), chiral aldehyde ((***S***)**-4A**) (107.7 mg, 0.2 mmol, 1.00 equiv.), and DBU (59.6 μL, 0.4 mmol, 2.0 equiv.) in DMSO (4.0 mL). The reaction mixture was allowed to stir for 24 s. The reaction mixture was quenched at 70 °C with 1 mL of 2 M FA then diluted with H_2O (5 mL). The aqueous layer was washed with DCM (5 × 5 mL). The combined organic layers were washed with H_2O (3 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. Isolated in 70% as a yellow solid after purification by normal phase column chromatography (7% EtOAc in DCM).

^{1}H NMR (d_6-DMSO, 500 MHz), δ 10.30 (s, 1H), 10.22 (s, 1H), 8.61 (s, 1H), 8.60 (s, 1H), 8.50 (s, 1H), 8.09–8.11 (m, 1H), 8.05 (d, *J* = 9.2 Hz, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 9.2 Hz, 1H), 7.20–7.45 (m, 11H), 6.96–7.05 (m, 4H), 6.60 (d, *J* = 8.2 Hz, 1H), 5.13 (s, 1H);

^{13}C NMR (d_6-DMSO, 125 MHz), δ 196.9, 154.1, 152.9, 152.3, 139.6, 139.5, 137.9, 136.9, 136.6, 133.3, 130.1, 130.0, 129.7, 128.9, 128.7, 128.5, 128.1, 127.2, 126.6, 124.5, 124.3, 124.0, 123.6, 122.7, 121.8, 120.2, 118.1, 117.6, 117.5, 117.4, 116.8, 115.7, 70.1;

HRMS (ESI): calcd. for $C_{35}H_{27}N_2O_4$ $[M+H]^+$: 539.1965. Found 539.1973.

Kinetic Data for DL, L, D Phenylalanine under Standard Reaction Conditions



a. DL-phenylalanine

Time	Recovery	^{13}C inc. (%)	*er*
2 h	85	17	78:22
6 h	84	41	84:16
24 h	57	72	92:8

b. L-phenylalanine

Time	Recovery	^{13}C inc. (%)	*er*
2 h	92	19	66:34
6 h	82	36	84:16
24 h	58	67	85:15

c. D-phenylalanine

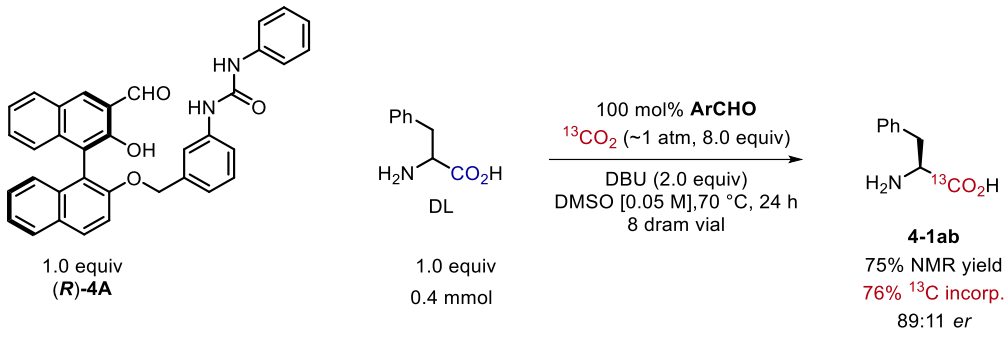
Time	Recovery	^{13}C inc. (%)	*er*
2 h	89	16	91:9
6 h	86	40	92:8
24 h	62	71	90:10

The experiment followed the general procedure A, using DL-^{12}C-phenylalanine, L-^{12}C-phenylalanine, D-^{12}C-phenylalanine respectively (8.3 mg, 0.05 mmol, 1.0 equiv.), chiral aldehyde ((***S*)-4A**) (53.9 mg, 0.1 mmol, 1.00 equiv.), DBU (14.9 µL, 0.1 mmol, 2.0 equiv.), and (DSS) in DMSO (1.0 mL). The reaction mixtures were sampled at 2 h, 6 h, and 24 h. To determine the percent recovery of the amino acid, a small aliquot (~10 µL) of the reaction was placed in 0.70 mL D_2O for calibrated ^{1}H NMR analysis, using DSS as the reference signal. To determine the % ^{13}C incorporation, a small aliquot of the reaction (~5 µL) was placed in 1.0 mL of

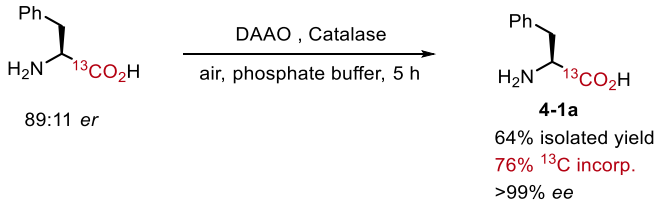
1:1 MeOH:H_2O/0.1% HCO_2H for LC-MS analysis. To determine *er*, a small aliquot of the reaction (~10 µL) was placed in 1.0 mL of 0.1% HCO_2H in H_2O for chiral HPLC analysis. Above is the representation of data for DL, L, D Phenylalanine at different time points.

Chiral Resolution of Amino Acids by DAAO

L-phenylalanine (4-1a)



Prepared according to the general procedure B from DL-^{12}C-phenylalanine (66.0 mg, 0.40 mmol, 1.0 equiv.), chiral aldehyde ((***R*)-4A**) (215.4 mg, 0.4 mmol, 1.00 equiv.), and DBU (119.2 µL, 0.8 mmol, 2.0 equiv.) in DMSO (8.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 75%. Isolated in 43% yield, *er:* 90:10, 76% ^{13}C incorporation (HRMS) as an off-white solid after purification by preparative-HPLC (Agilent Prep-C18 column, 2.5% MeOH in H_2O, 25 mL/min).



4-1a [C_1-^{13}C]phenylalanine *Step 2*. Adapted from a literature procedure.[211] In an 8-dram vial charged with a stir bar was added **4-1ab** (0.17 mmol). This was followed by the addition of catalase bovine liver (170 mg, 2000–5000 units/mg protein) and a solution of D-amino acid oxidases (DAAO) from porcine kidney (7.0 mg, ≥1.5 units/mg protein) in phosphate buffer (1.7 mL). The reaction mixture was stirred at room temperature for 5 h and then concentrated in vacuo. The aqueous layer was lyophilized subsequently to remove the water. Isolated in 64% yield (18.0 mg), 76%

^{13}C incorporation (HRMS), >99% *ee* after purification by preparative-HPLC (Agilent Prep-C18 column, 5% MeOH in H_2O, 30 mL/min) as a white solid.

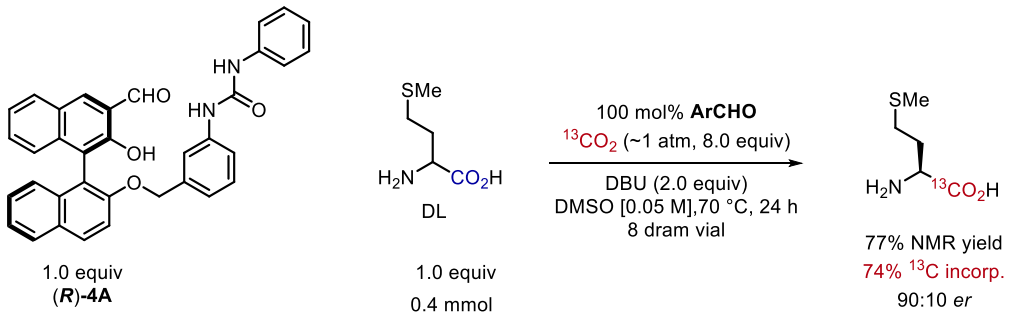
^{1}H NMR (D_2O, 500 MHz) δ 7.42–7.38 (m, 2H), 7.37–7.32 (m, 1H), 7.31–7.28 (m, 2H), 3.99–3.94 (m, 1H), 3.30–3.23 (m, 1H), 3.09 (dd, *J* = 14.5, 8.1 Hz, 1H);

^{13}C NMR (D_2O, 125 MHz) δ 173.6, 135.0, 129.4, 129.2, 127.8, 56.0 (d, *J* = 56.2 Hz), 36.3;

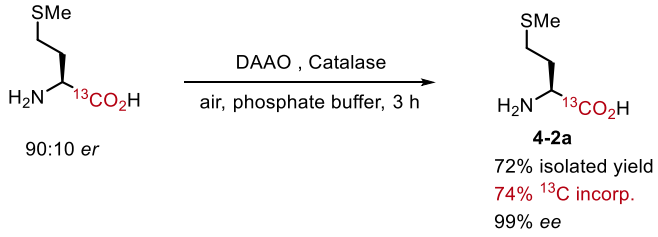
HRMS (ESI): calcd. for $C_8[^{13}C]H_{10}NO_2$ $[M-H]^-$: 165.0751. Found 165.0751;

Chiral HPLC: >99 *ee*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 9.6 min (major), t_r = 10.6 min (minor).

1. L-methionine (4-1a)



Prepared according to the general procedure B from DL-^{12}C-methionine (59.6 mg, 0.40 mmol, 1.0 equiv.), chiral aldehyde ((***R*)-4A**) (215.4 mg, 0.4 mmol, 1.00 equiv.), and DBU (119.2 μL, 0.8 mmol, 2.0 equiv.) in DMSO (8.0 mL). The reaction mixture was allowed to stir for 24 h. ^{1}H NMR yield: 77%. Isolated in 42% yield, *er:* 90:10, 74% ^{13}C incorporation (HRMS) as an off-white solid after purification by reverse phase column chromatography (5% MeCN in H_2O).



4-2a [C_1-^{13}C]-methionine *Step 2*. Adapted from a literature procedure.[211] In an 8-dram vial charged with a stir bar was added **4-1ab** (0.17 mmol). This was followed

by the addition of catalase bovine liver (170 mg, 2000–5000 units/mg protein) and a solution of D-amino acid oxidases (DAAO) from porcine kidney (7.0 mg, ≥1.5 units/mg protein) in phosphate buffer (1.7 mL). The reaction mixture was stirred at room temperature for 3 h and then concentrated in vacuo. The aqueous layer was lyophilized subsequently to remove the water. Isolated in 72% yield, 74% ^{13}C incorporation (HRMS), 99% *ee* after purification by reverse phase column chromatography (5% MeCN in H_2O).

^{1}H NMR (D_2O, 500 MHz) δ 3.91–3.94 (m, 1H), 2.71 (t, *J* = 7.4 Hz, 2H), 2.25–2.30 (m, 1H), 2.16 – 2.20 (m, 4H);

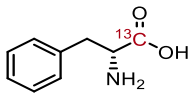
^{13}C NMR (D_2O, 125 MHz) δ 175.2, 54.7 (d, *J* = 53.3 Hz), 30.6, 29.8, 14.9;

HRMS (ESI): calcd. for $C_4[^{13}C]H_{10}NO_2S$ $[M-H]^-$: 149.0471. Found 149.0473;

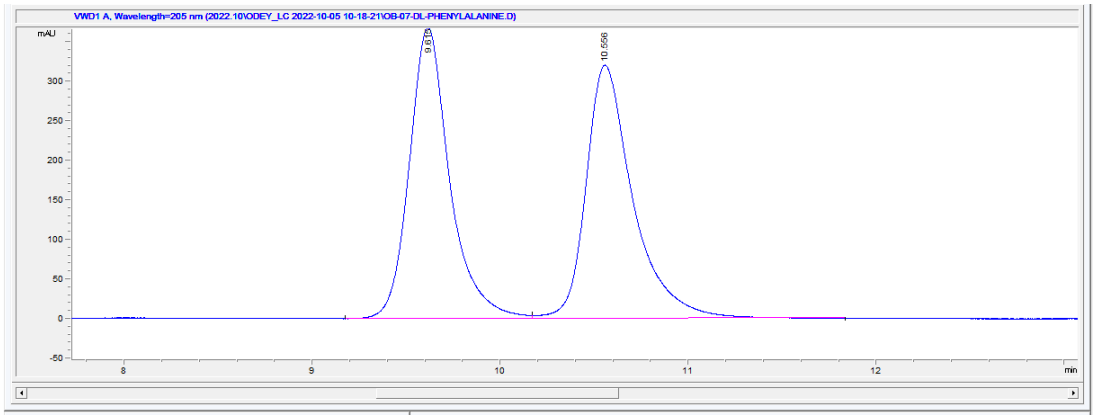
Chiral HPLC: 99 *ee*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.3 min (major), t_r = 13.2 min (minor).

Spectral images

A. HP LPLC Chromatograms



Chiral HPLC: 89:11 *er*. Determined on Astec Chirobiotic-T column (60% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 9.6 min (minor), t_r = 10.6 min (major).



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Sample Info	
Barcode	
Operator	Odey
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Sampling Rate	0.0012 min (0.072 sec), 18956 datapoints

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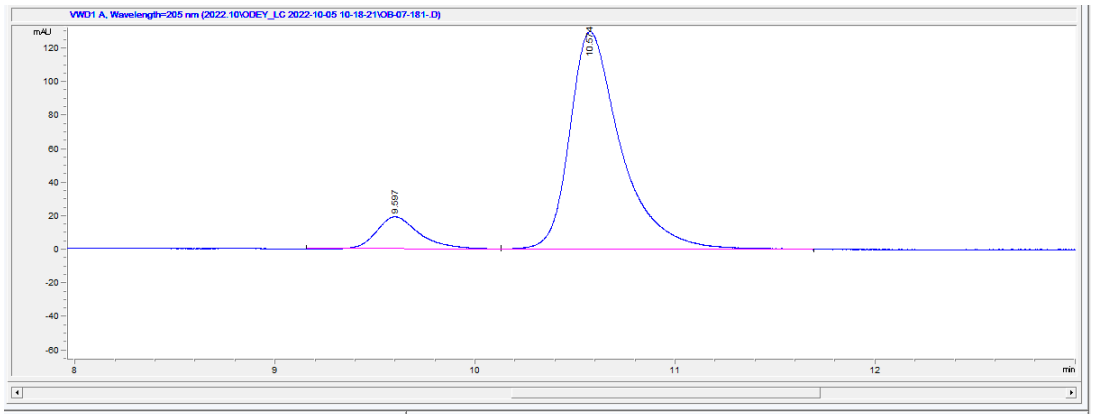

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Barcode	
Operator	Odey
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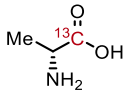
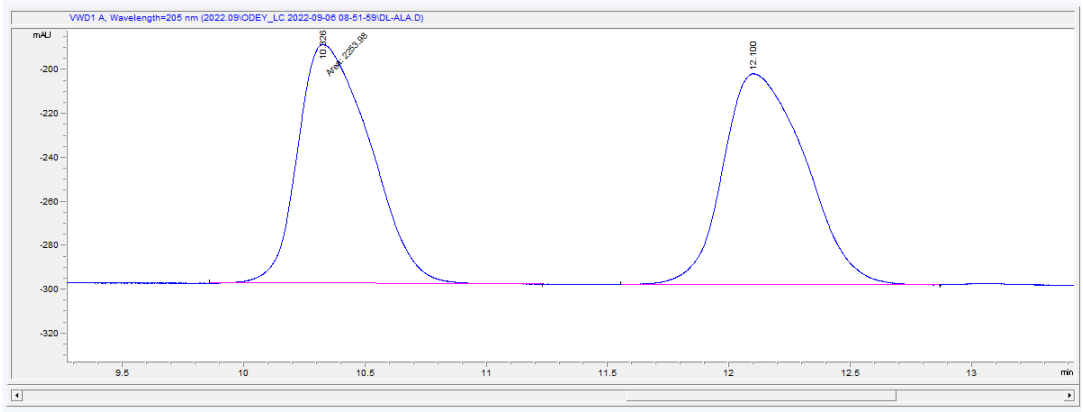


Chiral HPLC: 85:15 *er*. Determined on Astec Chirobiotic-T column (60% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.3 min (minor), t_r = 12.1 min (major).



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Sample Info	
Barcode	
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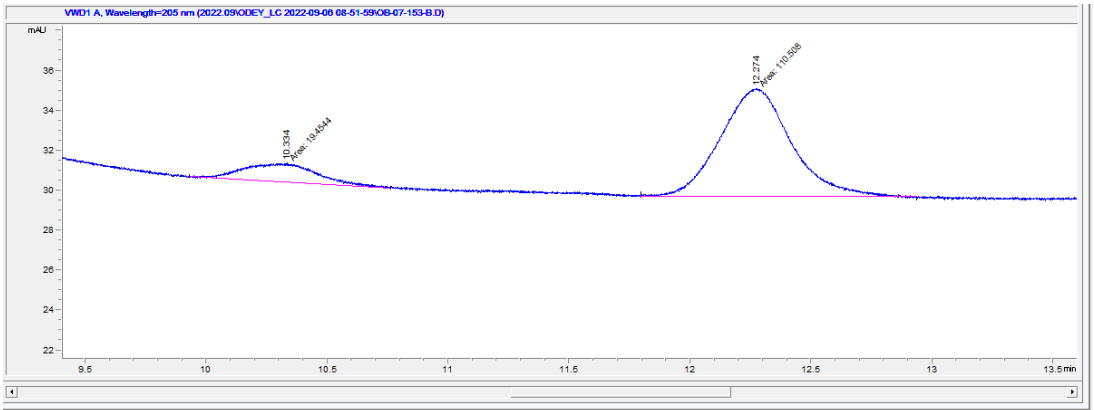

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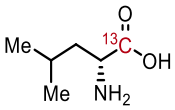
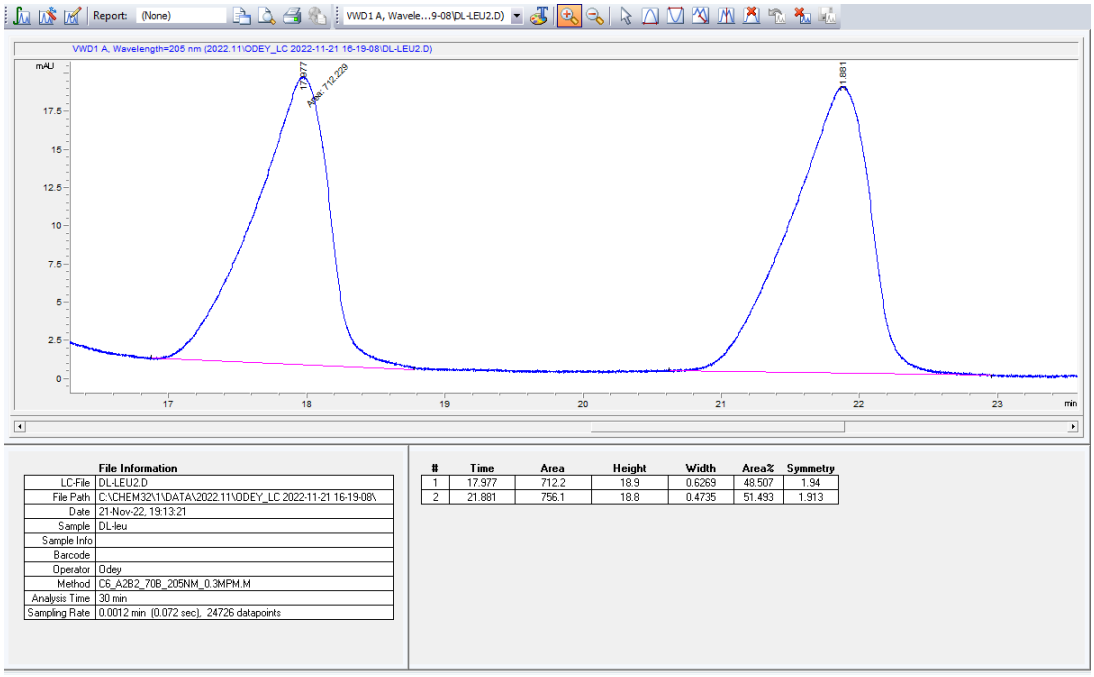
Barcode	
Operator	Odey
Method	C6_A2B2_60B_205NM_0.5MPM.M
Analysis Time	19.997 min
Sampling Rate	0.0012 min (0.072 sec), 16482 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.334	19.5	9.5E-1	0.3401	14.969	0.726
2	12.274	110.5	5.4	0.3414	85.031	1.002

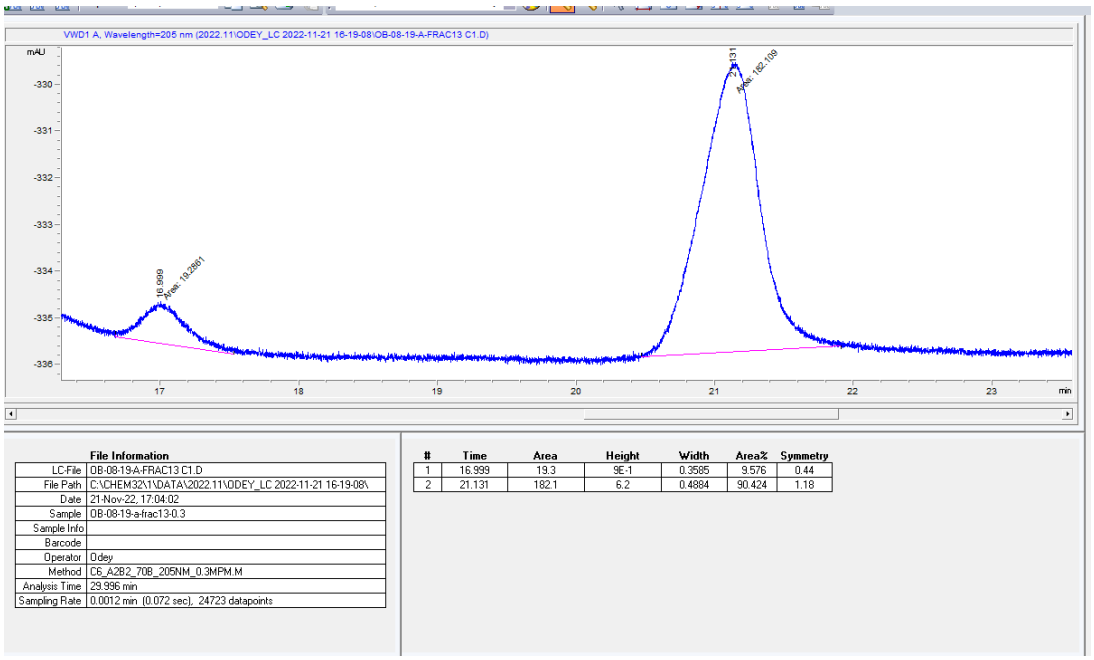


Chiral HPLC: 90:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.3 mL/min), t_r = 17.9 min (minor), t_r = 21.8 min (major).



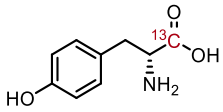
File Information	
LC-File	DL-LEU2.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-21 16-19-08\
Date	21-Nov-22, 19:13:21
Sample	DL-leu
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.3MPM.M
Analysis Time	30 min
Sampling Rate	0.0012 min (0.072 sec), 24726 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	17.977	712.2	18.9	0.6269	48.507	1.94
2	21.881	756.1	18.8	0.4735	51.493	1.913

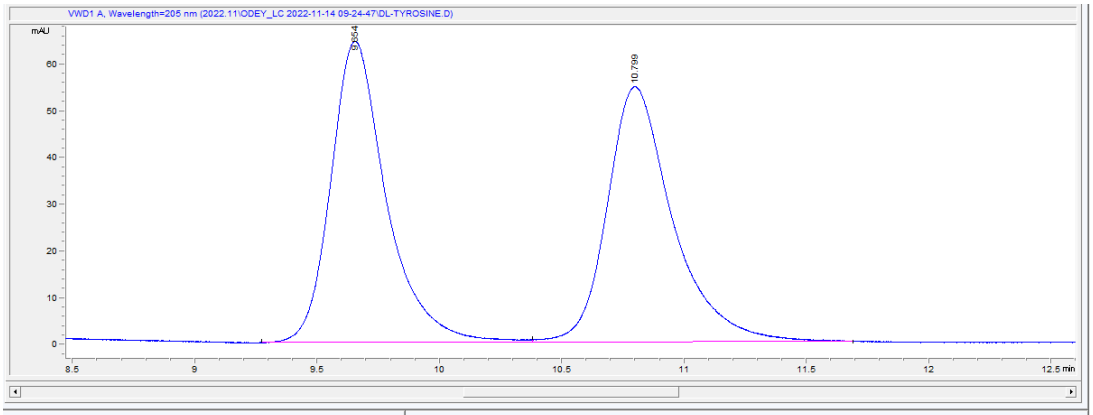


File Information	
LC-File	OB-08-19-A-FRAC13 C1.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-21 16-19-08\
Date	21-Nov-22, 17:04:02
Sample	OB-08-19-a-frac13-0.3
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.3MPM.M
Analysis Time	29.996 min
Sampling Rate	0.0012 min (0.072 sec), 24723 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	16.999	19.3	9E-1	0.3585	9.576	0.44
2	21.131	182.1	6.2	0.4884	90.424	1.18

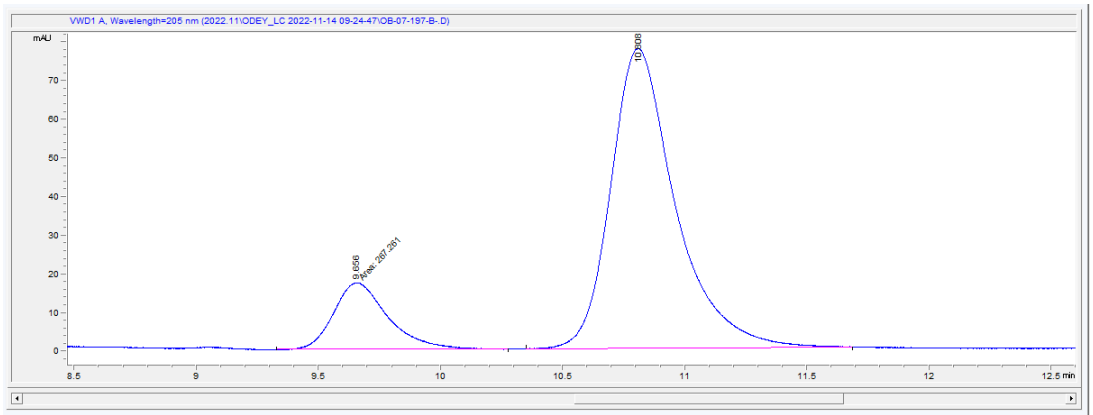


Chiral HPLC: 84:16 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 9.6 min (minor), t_r = 10.8 min (major).



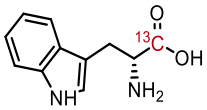
File Information	
LC-File	DL-TYROSINE.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-14 09-24-47\
Date	14-Nov-22, 09:26:21
Sample	DL-tyrosine
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	19.997 min
Sampling Rate	0.0012 min (0.072 sec), 16482 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	9.654	1018.6	64.4	0.2332	49.983	0.723
2	10.799	1019.3	54.6	0.2718	50.017	0.699

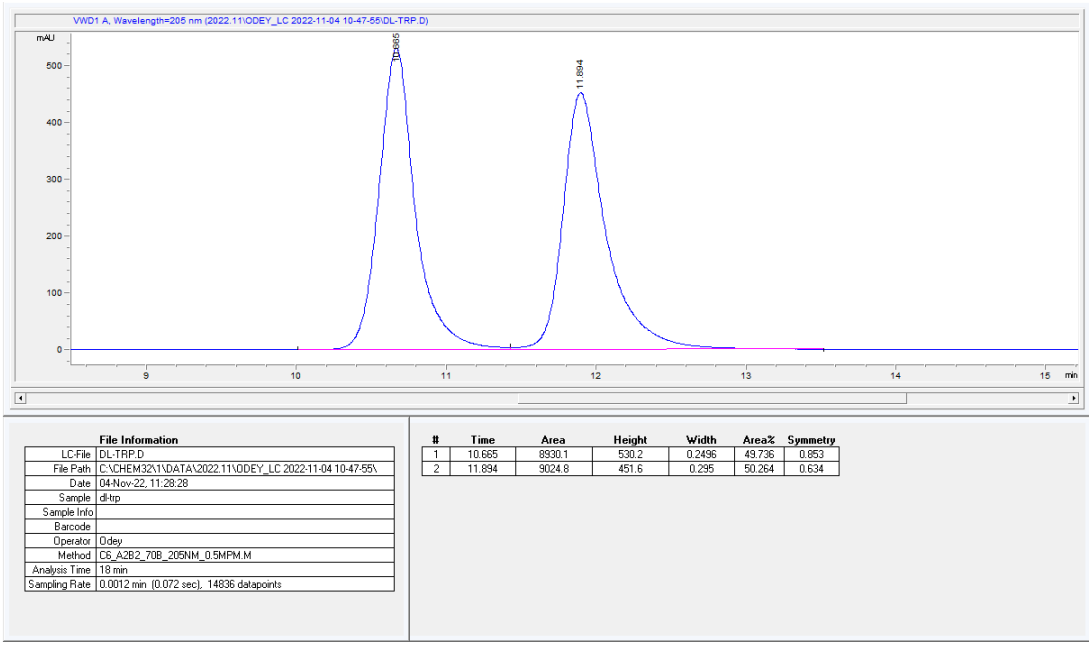


File Information	
LC-File	OB-07-197-B-.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-14 09-24-47\
Date	14-Nov-22, 10:09:55
Sample	OB-07-197-b-
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	15.999 min
Sampling Rate	0.0012 min (0.072 sec), 13187 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	9.656	267.3	17.3	0.258	15.701	0.715
2	10.808	1434.9	77.6	0.2741	84.299	0.68

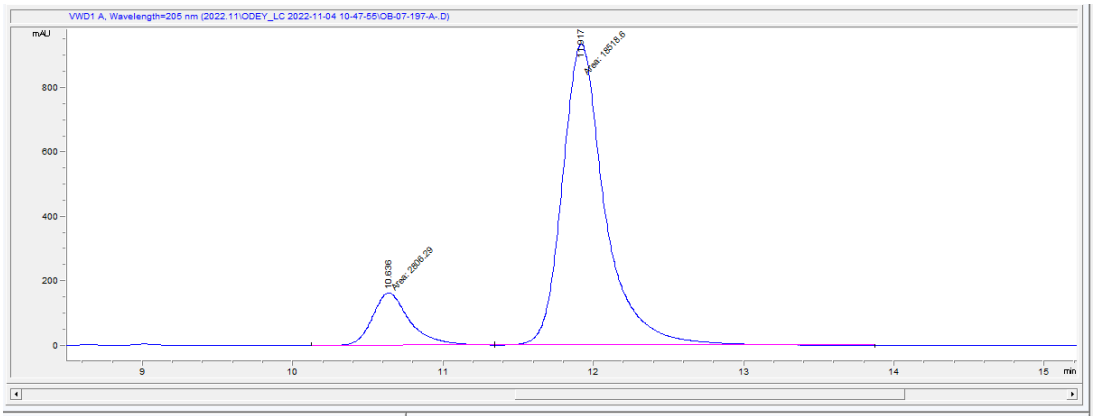


Chiral HPLC: 87:14 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.6 min (minor), t_r = 11.9 min (major).



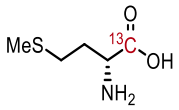
File Information	
LC-File	DL-TRP.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-04 10-47-55\
Date	04-Nov-22, 11:28:28
Sample	dl-trp
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	18 min
Sampling Rate	0.0012 min (0.072 sec), 14836 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.665	8930.1	530.2	0.2496	49.736	0.853
2	11.894	9024.8	451.6	0.295	50.264	0.634

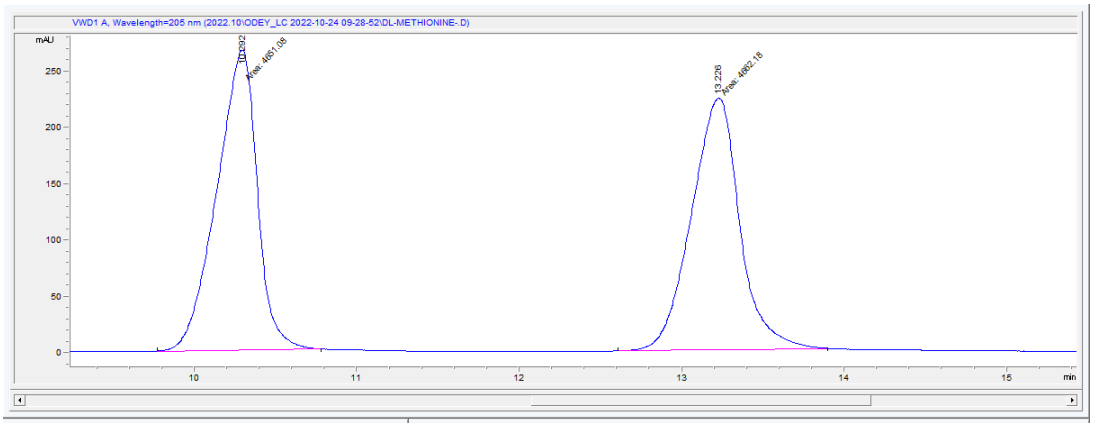


File Information	
LC-File	OB-07-197-A-.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-04 10-47-55\
Date	04-Nov-22, 11:08:52
Sample	ob-07-197-A-
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	17.996 min
Sampling Rate	0.0012 min (0.072 sec), 14833 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.636	2806.3	163	0.2869	13.160	0.753
2	11.917	18518.6	935.5	0.3299	86.840	0.756

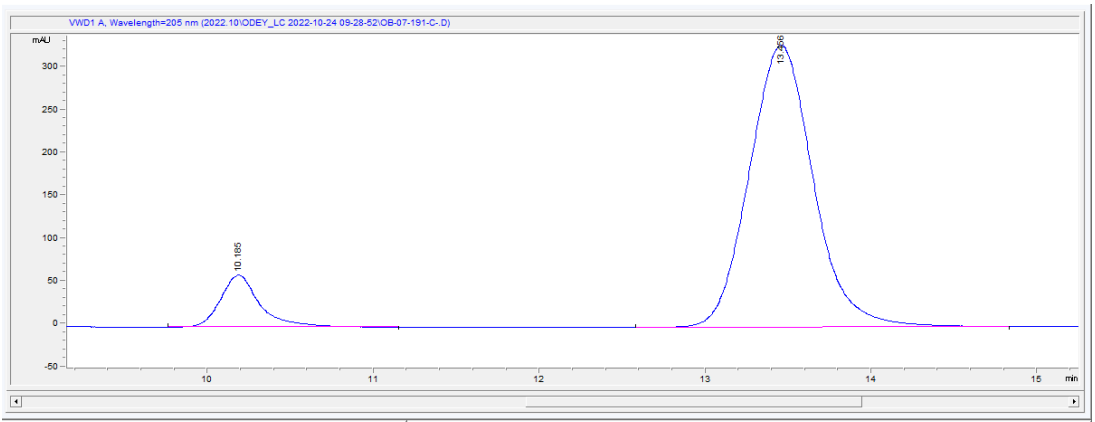


Chiral HPLC: 90:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.3 min (minor), t_r = 13.2 min (major).



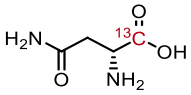
File Information	
LC-File	DL-METHIONINE-.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-24 09-28-52\
Date	24-Oct-22, 10:12:07
Sample	DL-Methionine-
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	18.996 min
Sampling Rate	0.0012 min (0.072 sec), 15657 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.292	4651.1	266.7	0.2907	49.940	1.591
2	13.226	4662.2	223.9	0.3471	50.060	1.236

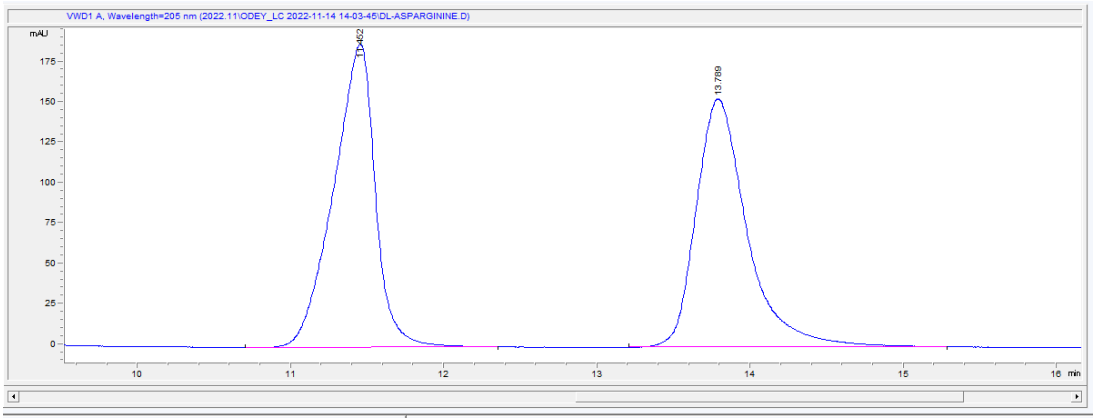


File Information	
LC-File	OB-07-191-C-.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-24 09-28-52\
Date	24-Oct-22, 10:33:19
Sample	ob-07-191-C-
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	18.997 min
Sampling Rate	0.0012 min (0.072 sec), 15658 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.185	1013.9	60.7	0.2436	10.286	0.766
2	13.456	8842.5	329.5	0.4106	89.714	0.925

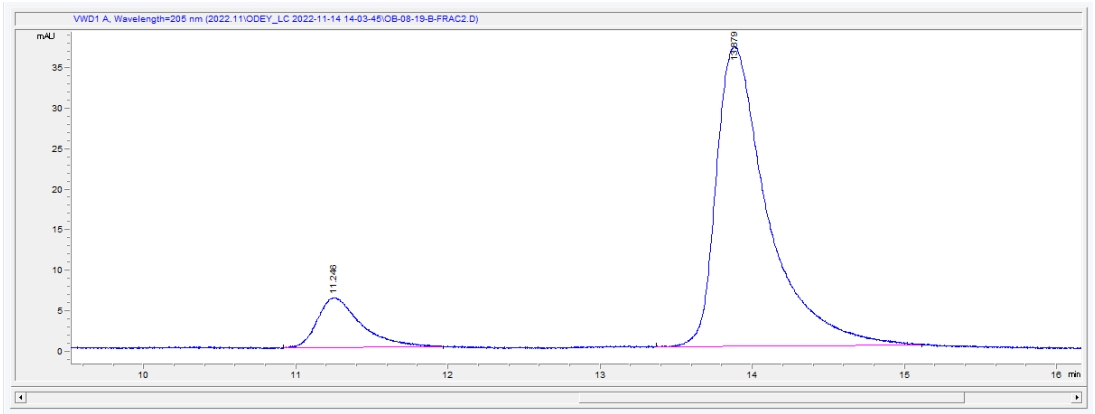


Chiral HPLC: 87:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 11.5 min (minor), t_r = 13.8 min (major).



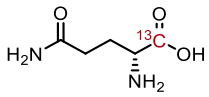
File Information	
LC-File	DL-ASPARGININE.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-14 14-03-45\
Date	14-Nov-22, 14:05:23
Sample	DL-Asparginine
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	17.996 min
Sampling Rate	0.0012 min (0.072 sec), 14833 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	11.452	3560.6	187.9	0.2749	49.951	1.641
2	13.789	3567.6	153.8	0.3434	50.049	0.706

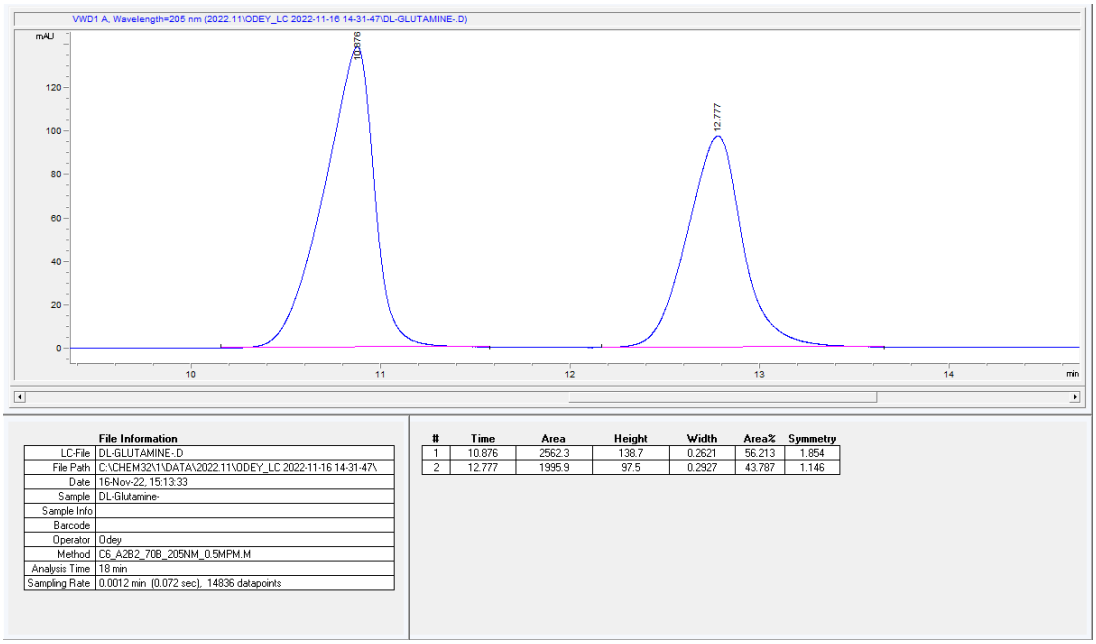


File Information	
LC-File	OB-08-19-B-FRAC2.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-14 14-03-45\
Date	14-Nov-22, 14:25:15
Sample	ob-08-19-b-frac2
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	18 min
Sampling Rate	0.0012 min (0.072 sec), 14836 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	11.246	123.8	6.2	0.239	12.388	0.58
2	13.879	875.4	37.1	0.3156	87.612	0.5

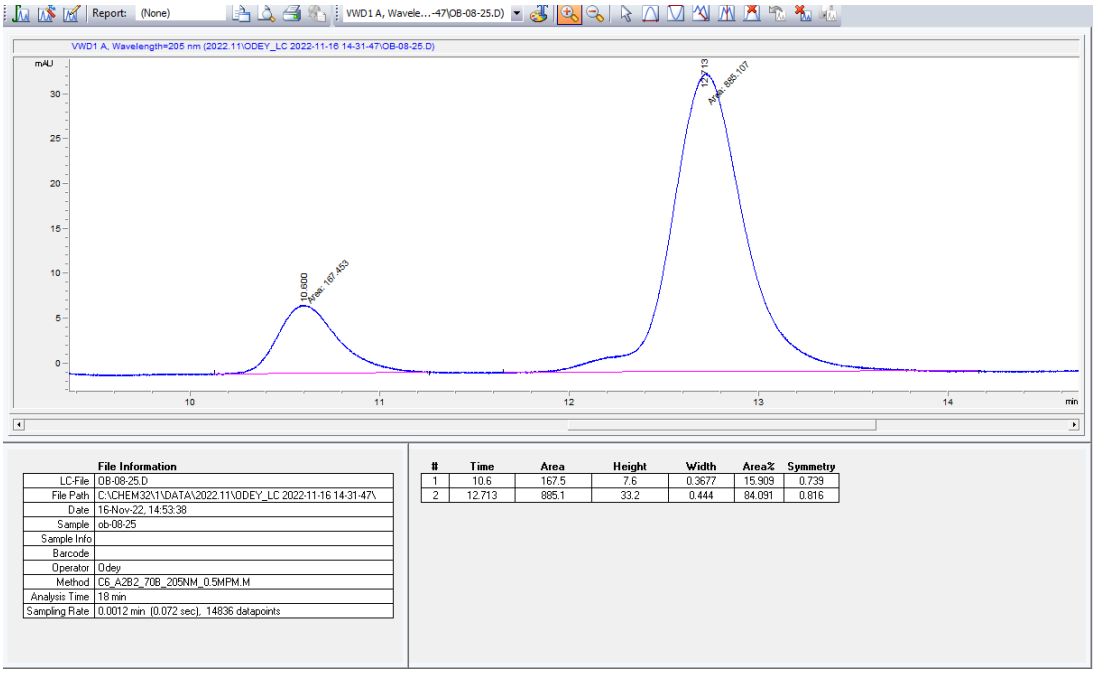


Chiral HPLC: 84:16 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 11.5 min (minor), t_r = 13.8 min (major).



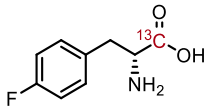
File Information	
LC-File	DL-GLUTAMINE- D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-16 14-31-47\
Date	16-Nov-22, 15:13:33
Sample	DL-Glutamine-
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	18 min
Sampling Rate	0.0012 min (0.072 sec), 14836 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.876	2562.3	138.7	0.2621	56.213	1.854
2	12.777	1995.9	97.5	0.2927	43.787	1.146

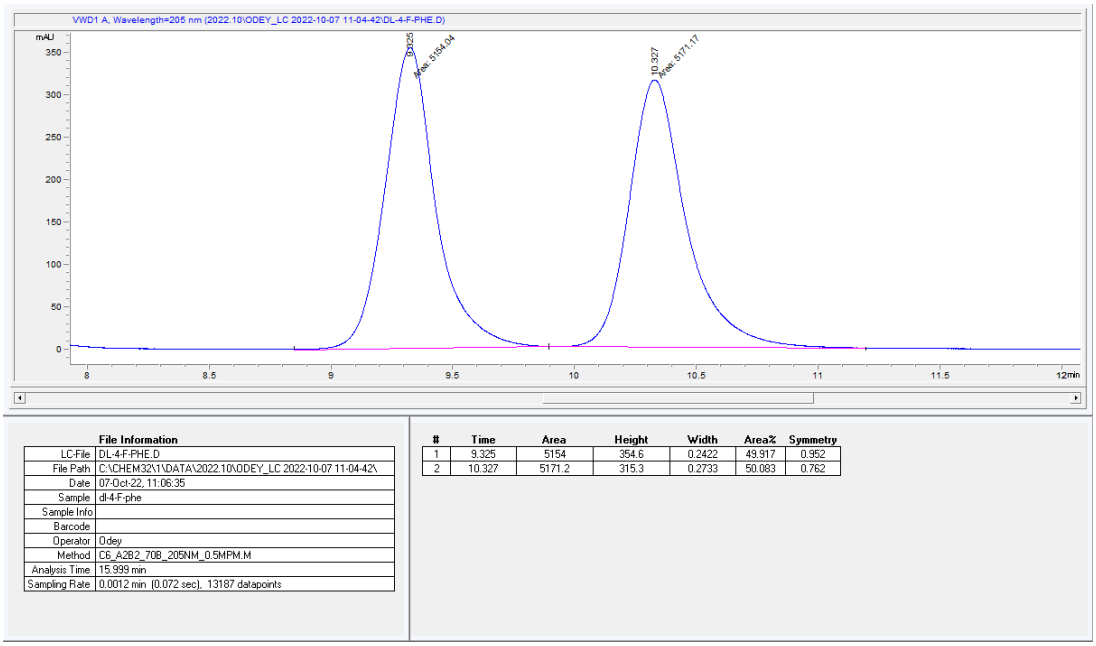


File Information	
LC-File	OB-08-25.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-16 14-31-47\
Date	16-Nov-22, 14:53:38
Sample	ob-08-25
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	18 min
Sampling Rate	0.0012 min (0.072 sec), 14836 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.6	167.5	7.6	0.3677	15.909	0.739
2	12.713	885.1	33.2	0.444	84.091	0.816

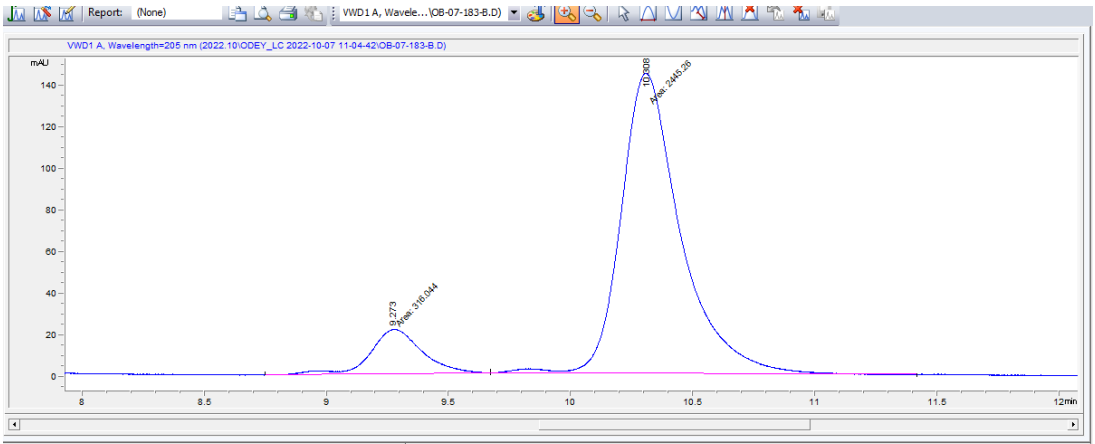


Chiral HPLC: 89:11 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 9.3 min (minor), t_r = 10.3 min (major).



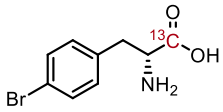
File Information	
LC-File	DL-4-F-PHE.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-07 11-04-42\
Date	07-Oct-22, 11:06:35
Sample	dl-4-F-phe
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	15.999 min
Sampling Rate	0.0012 min (0.072 sec), 13187 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	9.325	5154	354.6	0.2422	49.917	0.952
2	10.327	5171.2	315.3	0.2733	50.083	0.762

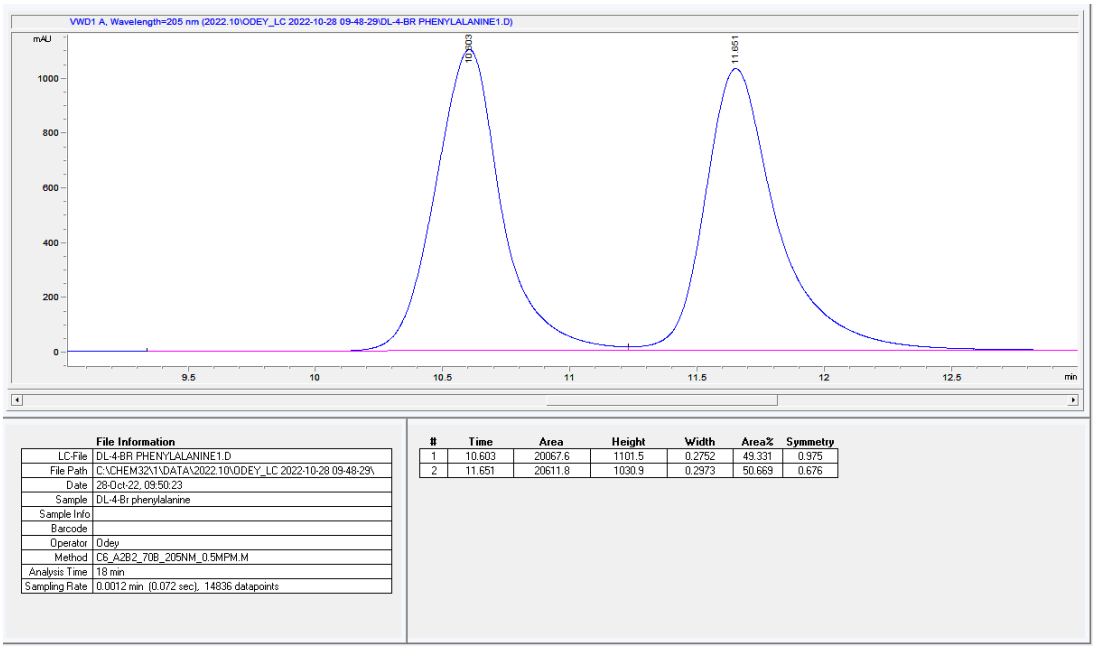


File Information	
LC-File	OB-07-183-B.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-07 11-04-42\
Date	07-Oct-22, 11:24:43
Sample	ob-07-183-b
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	15.998 min
Sampling Rate	0.0012 min (0.072 sec), 13186 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	9.273	316	21.4	0.2463	11.445	0.819
2	10.308	2445.3	144.3	0.2823	88.555	0.711

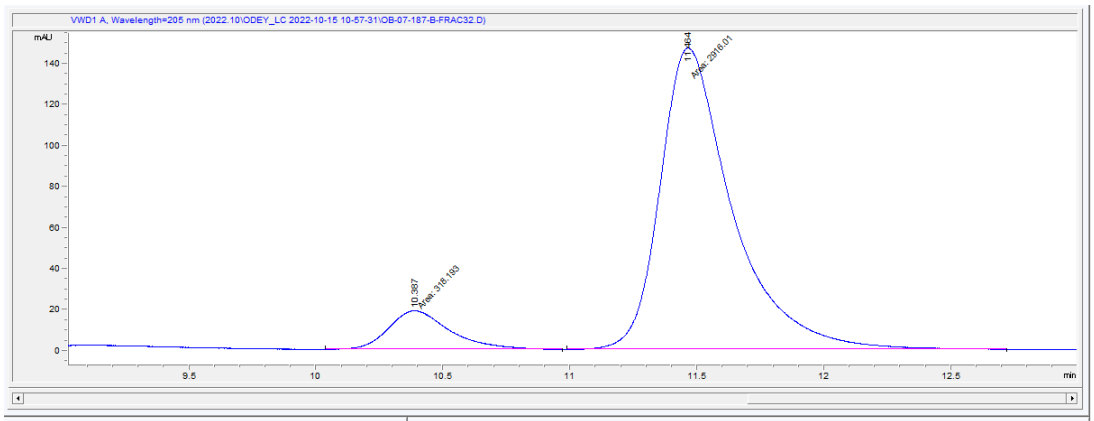


Chiral HPLC: 90:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.6 min (minor), t_r = 11.7 min (major).



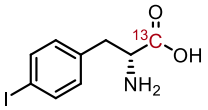
File Information	
LC-File	DL-4-BR PHENYLALANINE1.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-28 09-48-29\
Date	28-Oct-22, 09:50:23
Sample	DL-4-Br phenylalanine
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	18 min
Sampling Rate	0.0012 min (0.072 sec), 14836 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.603	20067.6	1101.5	0.2752	49.331	0.975
2	11.651	20611.8	1030.9	0.2973	50.669	0.676

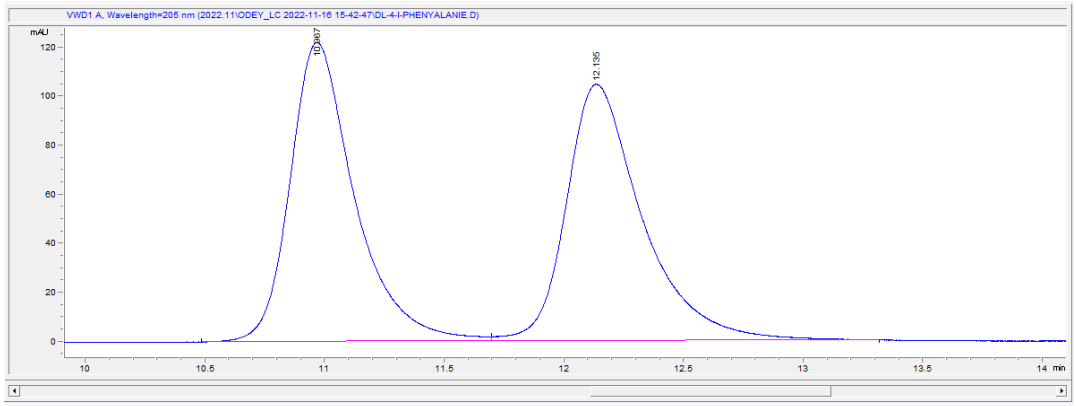


File Information	
LC-File	OB-07-187-B-FRAC32.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-15 10-57-31\
Date	15-Oct-22, 13:46:35
Sample	OB-07-187-b-frac32
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	12.996 min
Sampling Rate	0.0012 min (0.072 sec), 10712 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.387	318.2	18.8	0.2817	9.838	0.713
2	11.464	2916	147.1	0.3304	90.162	0.606

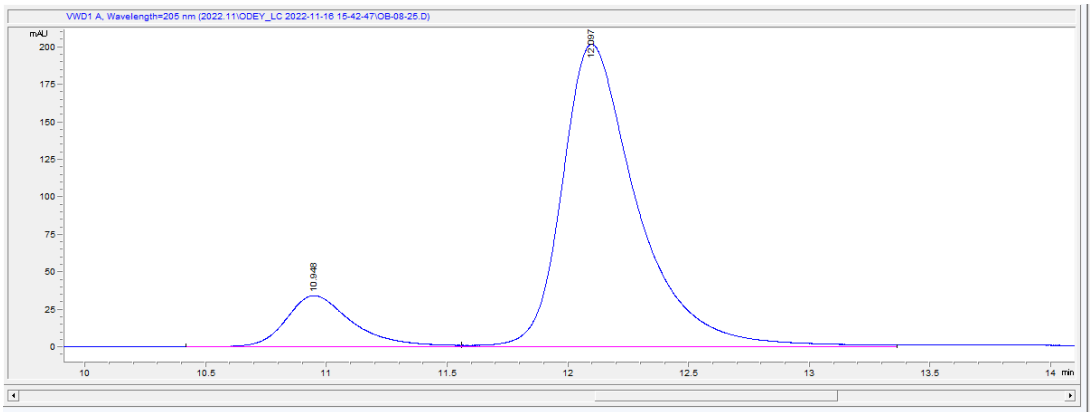


Chiral HPLC: 87:13 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.9 min (minor), t_r = 12.1 min (major).



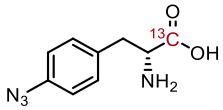
File Information	
LC-File	DL-4-I-PHENYALANIE.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-16 15-42-47\
Date	16-Nov-22, 15:44:42
Sample	DL-4-I-phenyalanie
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	18 min
Sampling Rate	0.0012 min (0.072 sec), 14836 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.967	2298.1	122	0.278	49.742	0.663
2	12.135	2321.9	104.7	0.3192	50.258	0.624

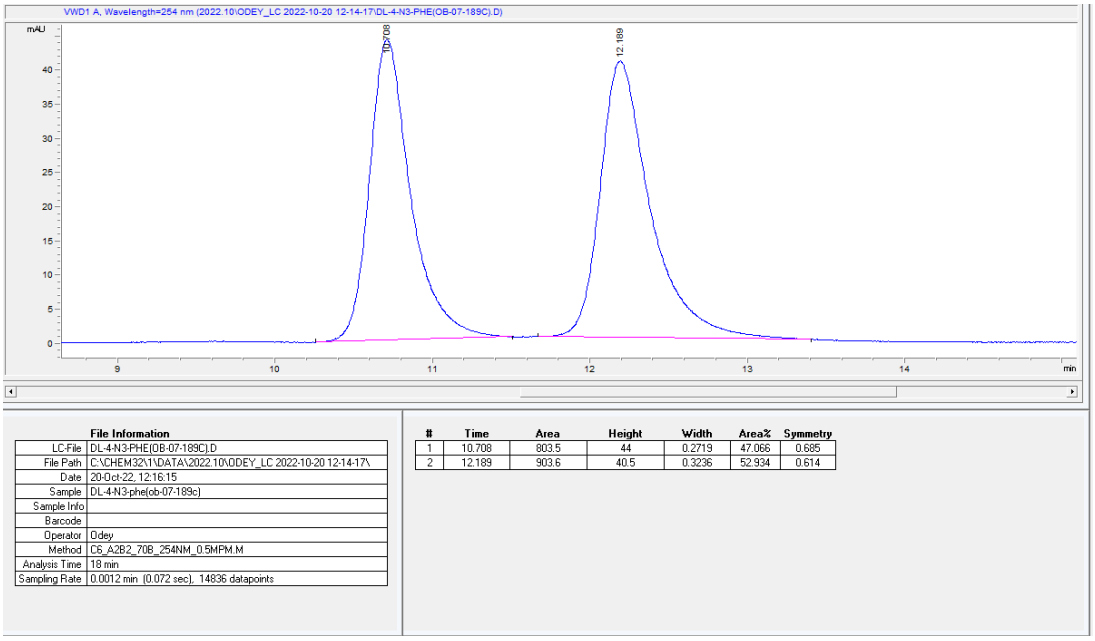


File Information	
LC-File	OB-08-25.D
File Path	C:\CHEM32\1\DATA\2022.11\ODEY_LC 2022-11-16 15-42-47\
Date	16-Nov-22, 16:04:42
Sample	ob-07-187-b
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_205NM_0.5MPM.M
Analysis Time	17.996 min
Sampling Rate	0.0012 min (0.072 sec), 14833 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.948	652.8	34.1	0.2784	13.032	0.681
2	12.097	4356.6	201.7	0.3154	86.968	0.603

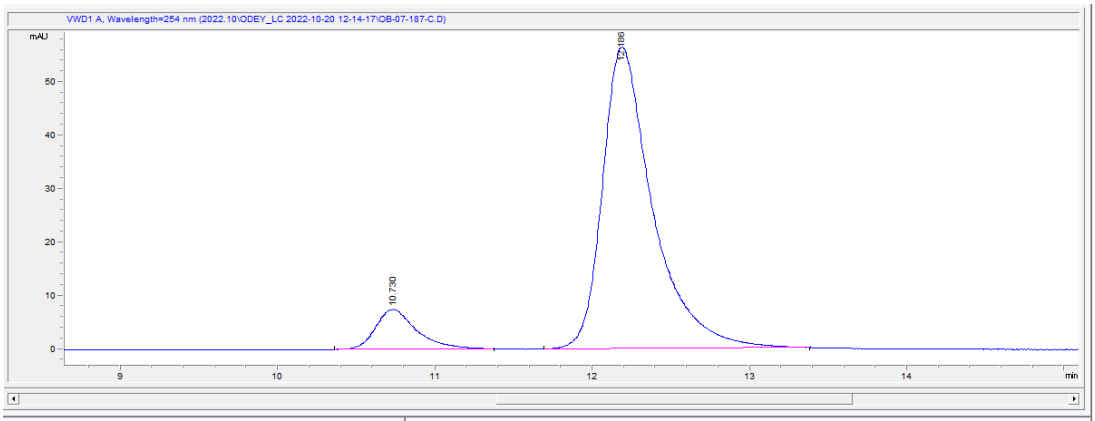


Chiral HPLC: 90:10 *er*. Determined on Astec Chirobiotic-T column (70% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.7 min (minor), t_r = 12.2 min (major).



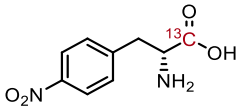
File Information	
LC-File	DL-4-N3-PHE(OB-07-189C).D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-20 12-14-17\
Date	20-Oct-22, 12:16:15
Sample	DL-4-N3-phe(ob-07-189c)
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_254NM_0.5MPM.M
Analysis Time	18 min
Sampling Rate	0.0012 min (0.072 sec), 14836 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.708	803.5	44	0.2719	47.066	0.685
2	12.189	903.6	40.5	0.3236	52.934	0.614

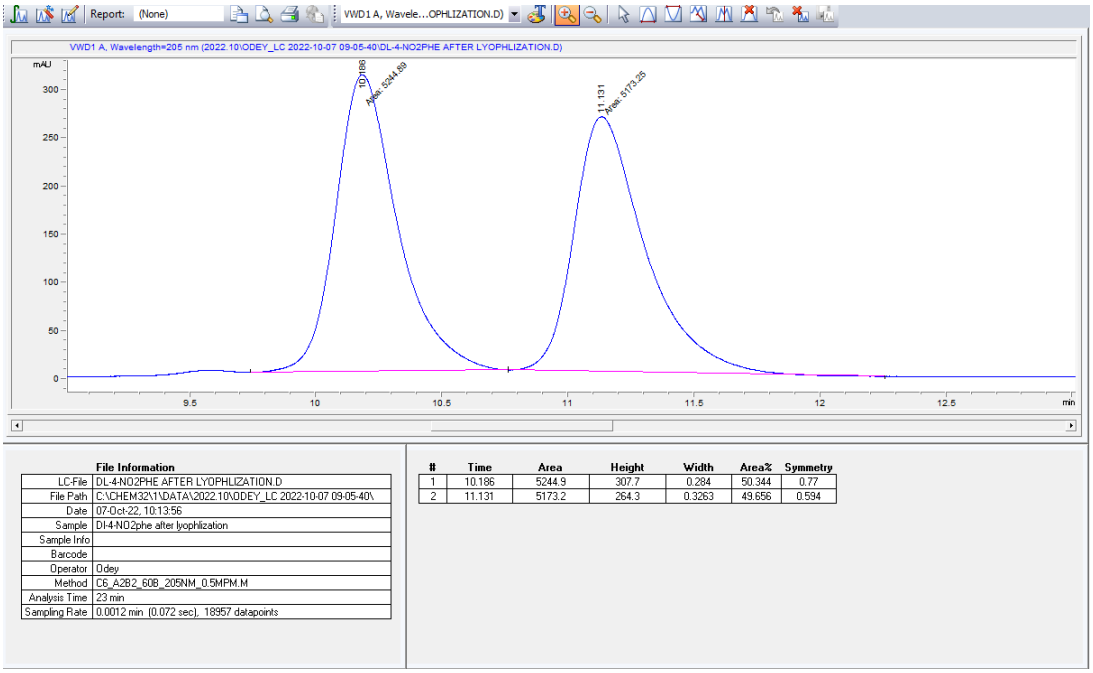


File Information	
LC-File	OB-07-187-C.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-20 12-14-17\
Date	20-Oct-22, 12:36:22
Sample	OB-07-187-c
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_70B_254NM_0.5MPM.M
Analysis Time	18.997 min
Sampling Rate	0.0012 min (0.072 sec), 15658 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.73	136.6	7.5	0.2251	9.780	0.716
2	12.186	1260.1	56.5	0.3265	90.220	0.586

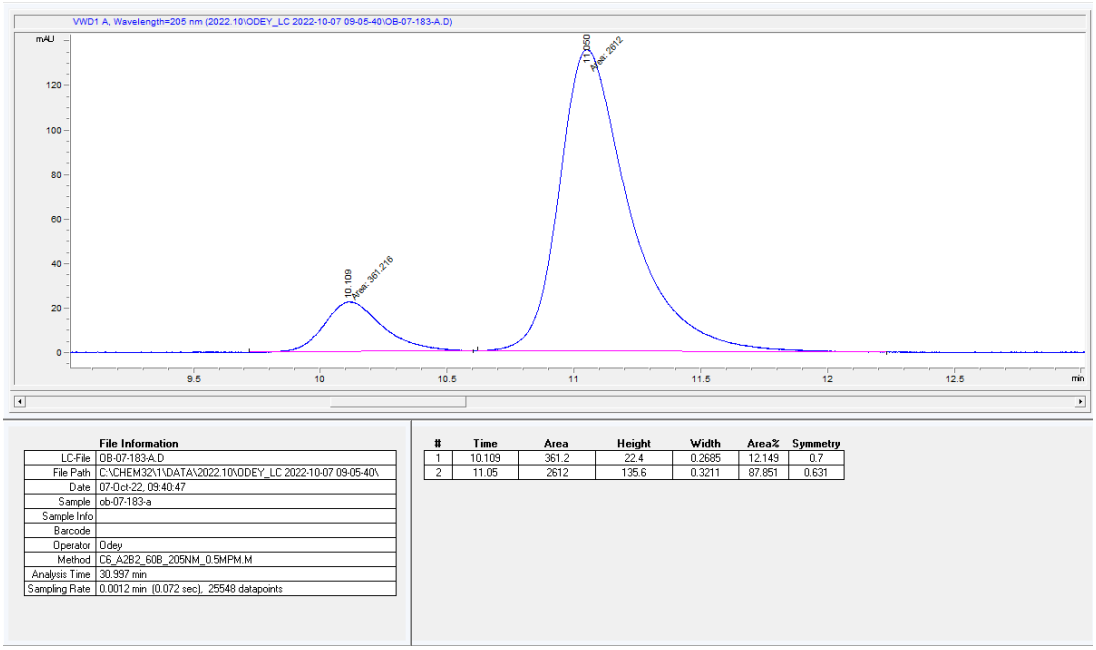


Chiral HPLC: 88:12 *er*. Determined on Astec Chirobiotic-T column (60% MeOH in H_2O with 0.02% HCO_2H, 0.5 mL/min), t_r = 10.2 min (minor), t_r = 11.1 min (major).



File Information	
LC-File	DL-4-NO2PHE AFTER LYOPHLIZATION.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-07 09-05-40\
Date	07-Oct-22, 10:13:56
Sample	DI-4-NO2phe after lyophlization
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_60B_205NM_0.5MPM.M
Analysis Time	23 min
Sampling Rate	0.0012 min (0.072 sec), 18957 datapoints

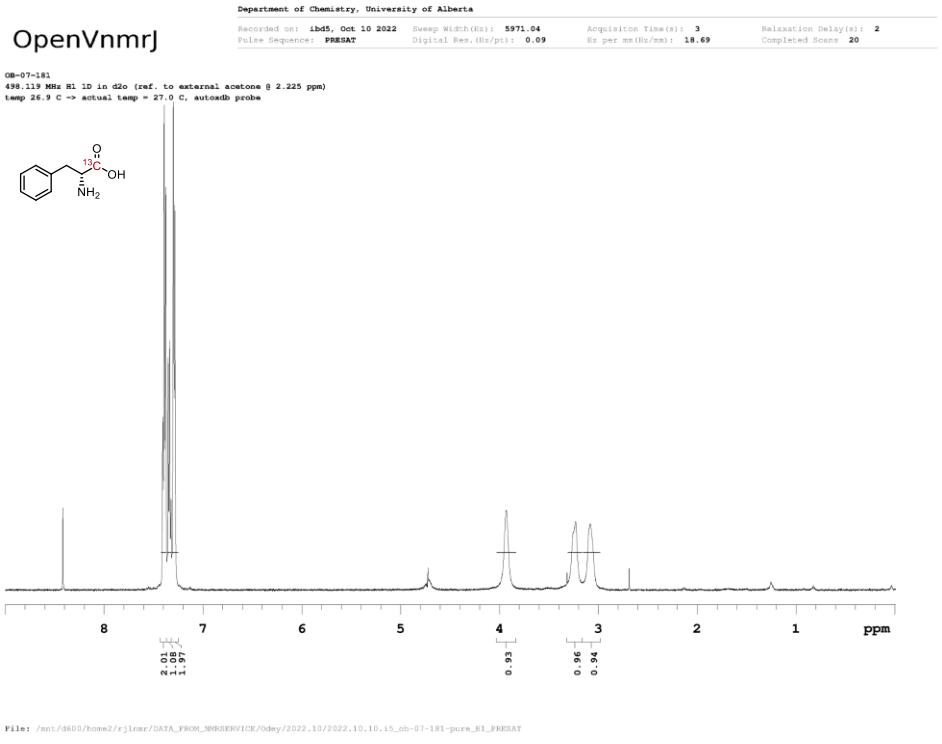
#	Time	Area	Height	Width	Area%	Symmetry
1	10.186	5244.9	307.7	0.284	50.344	0.77
2	11.131	5173.2	264.3	0.3263	49.656	0.594

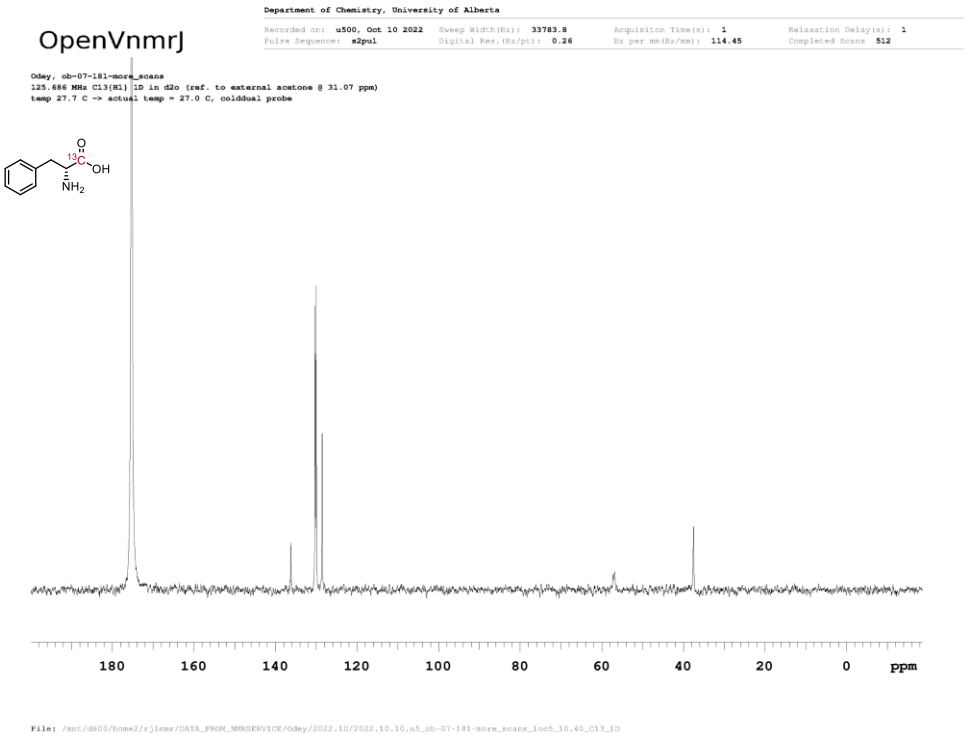


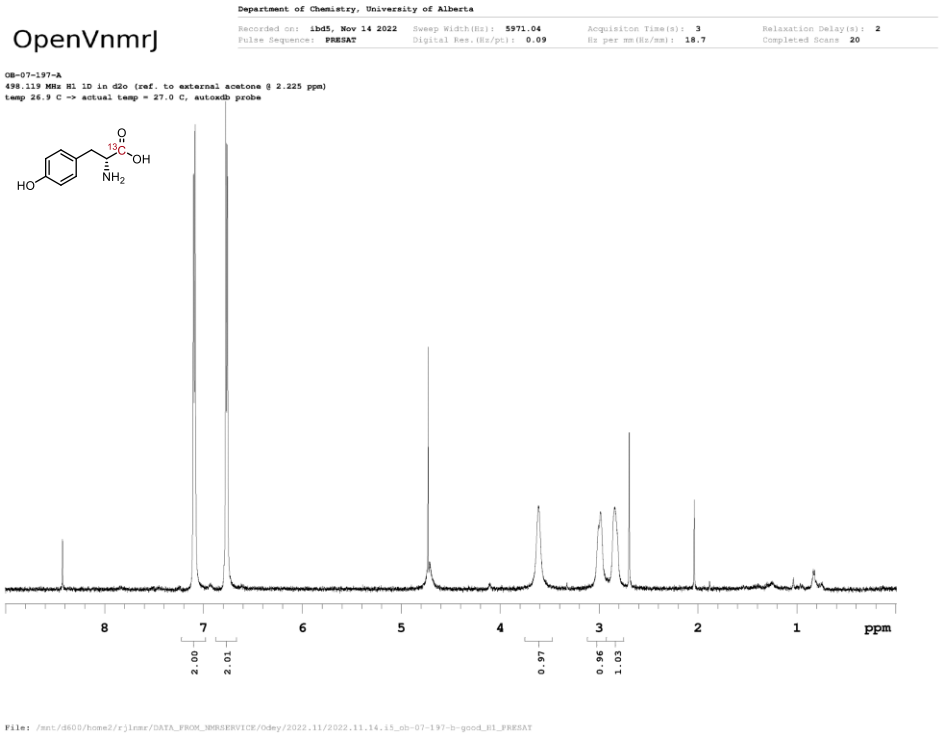
File Information	
LC-File	OB-07-183-A.D
File Path	C:\CHEM32\1\DATA\2022.10\ODEY_LC 2022-10-07 09-05-40\
Date	07-Oct-22, 09:40:47
Sample	ob-07-183-a
Sample Info	
Barcode	
Operator	Odey
Method	C6_A2B2_60B_205NM_0.5MPM.M
Analysis Time	30.997 min
Sampling Rate	0.0012 min (0.072 sec), 25548 datapoints

#	Time	Area	Height	Width	Area%	Symmetry
1	10.109	361.2	22.4	0.2685	12.149	0.7
2	11.05	2612	135.6	0.3211	87.851	0.631

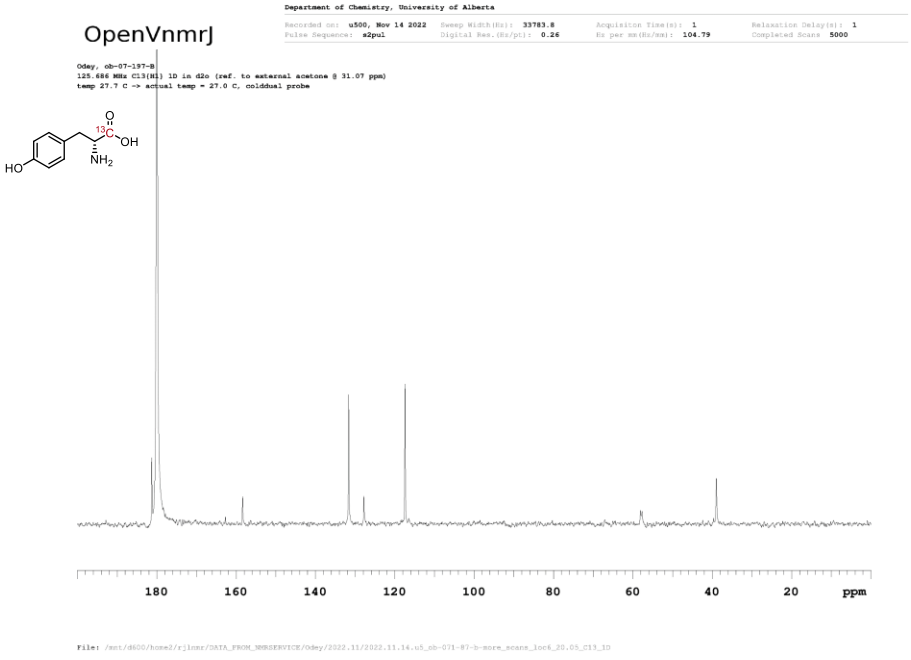
B. ^{1}H and ^{13}C spectra



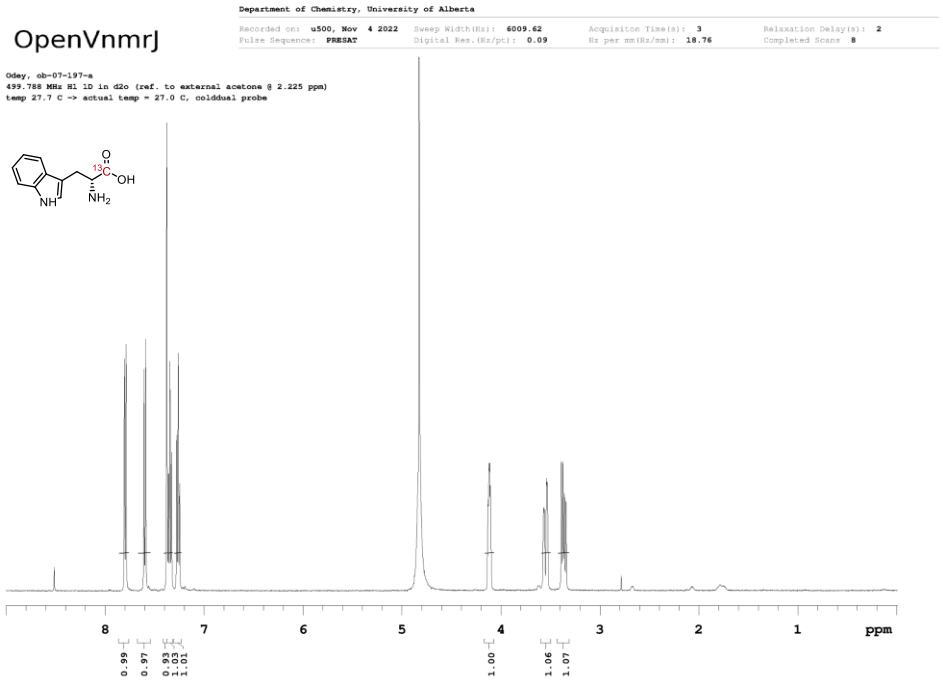




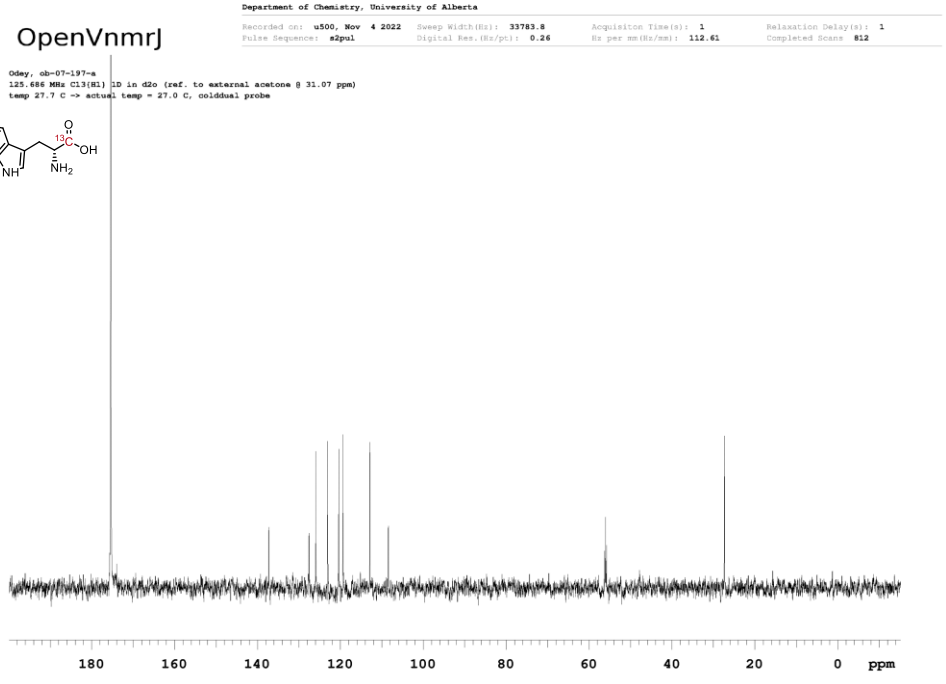
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.14.15_ob-07-197-b-good_H1_PRESAT



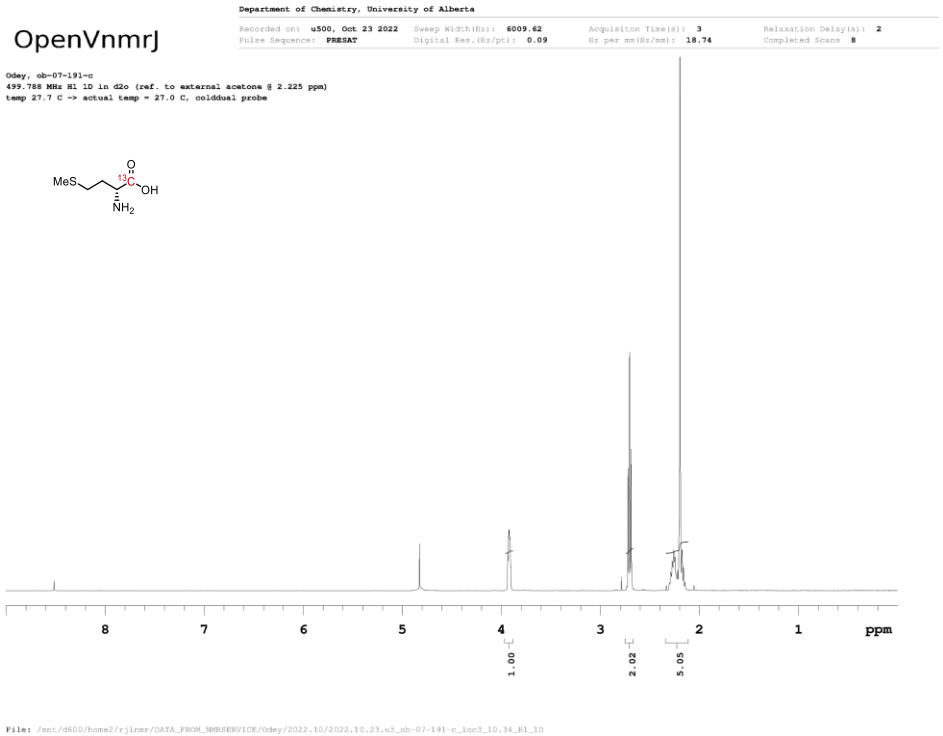
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.14.u5_ob-071-87-b-more_scans_loc6_20.05_C13_1D

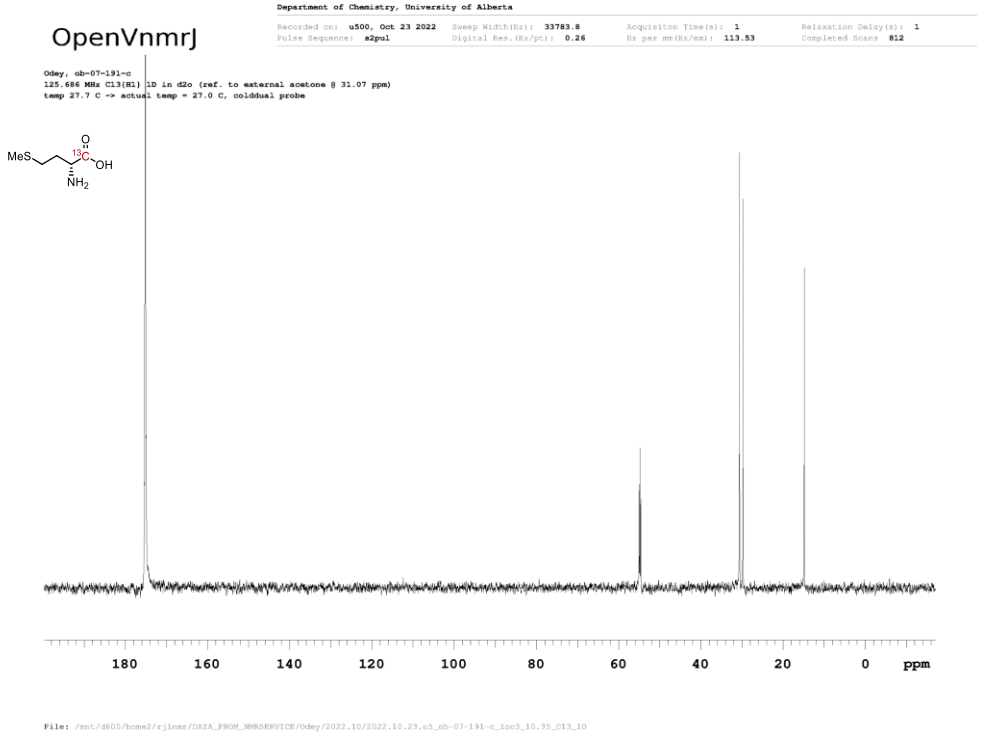


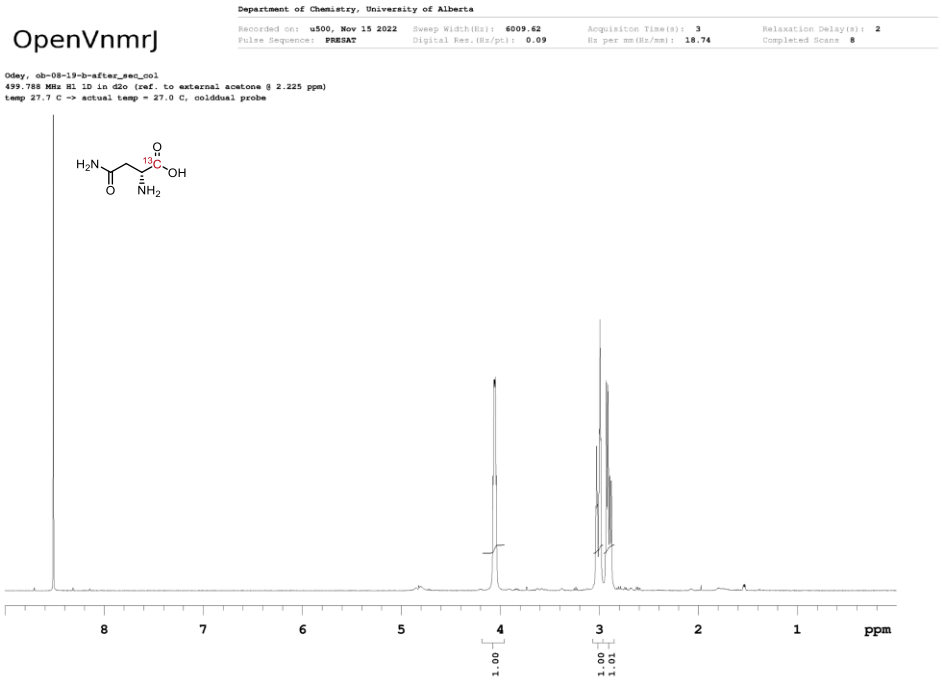
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.04.u5_ob-07-197-a_loc5_09.41_H1_1D



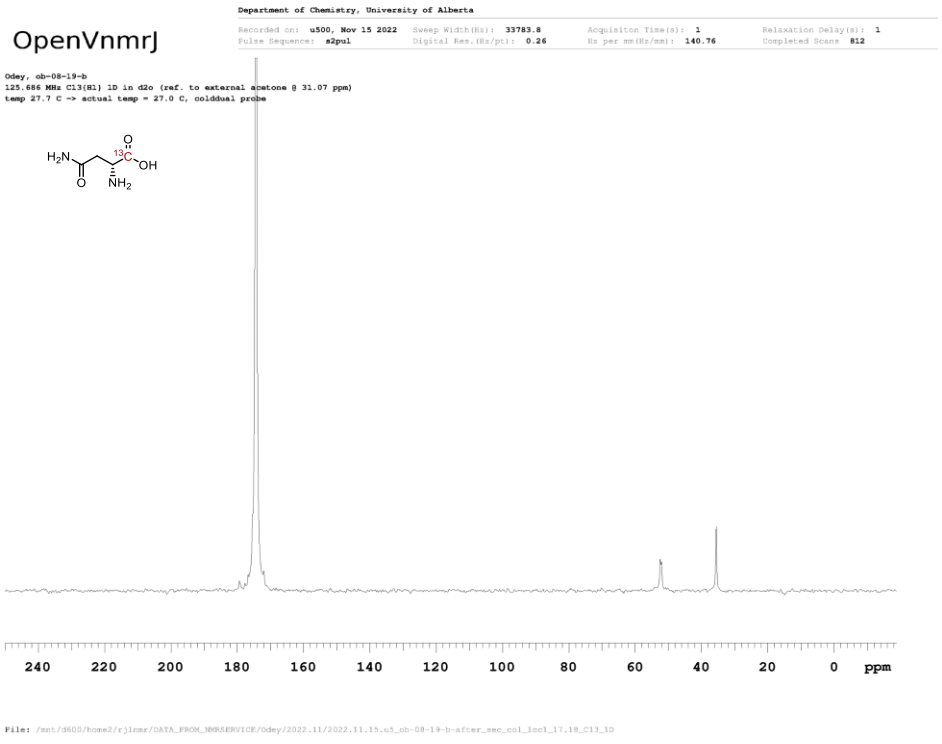
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.04.u5_ob-07-197-a_loc5_09.42_C13_1D


OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: u500, Oct 23 2022 Sweep Width(Hz): 6009.62 Acquisiton Time(s): 3 Relaxation Delay(s): 2
Pulse Sequence: PRESAT Digital Res.(Hz/pt): 0.09 Hz per mm(Hz/mm): 18.74 Completed Scans 8
Odey, ob-07-191-c
499.788 MHz H1 1D in d2o (ref. to external acetone @ 2.225 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
MeS
13C
OH
NH2
8
7
6
5
4
3
2
1
ppm
1.00
2.02
5.05
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.23.u5_ob-07-191-c_loc3_10.34_H1_1D

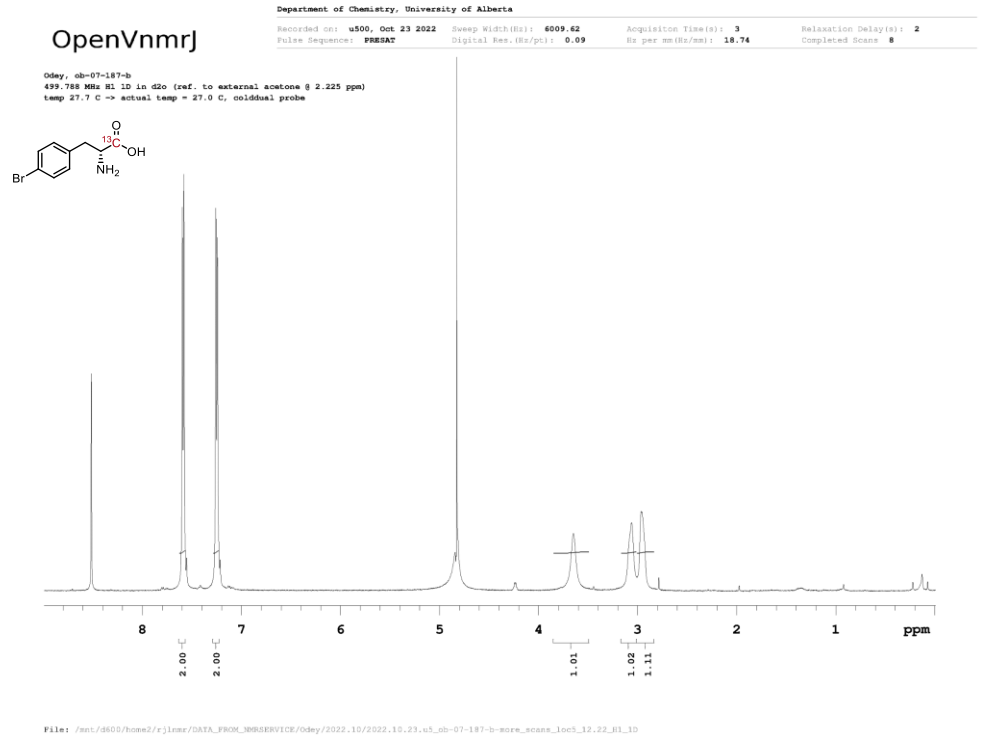

OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: u500, Oct 23 2022 Sweep Width(Hz): 33783.8 Acquisiton Time(s): 1 Relaxation Delay(s): 1
Pulse Sequence: s2pul Digital Res.(Hz/pt): 0.26 Hz per mm(Hz/mm): 113.53 Completed Scans 812
Odey, ob-07-191-c
125.686 MHz C13{H1} 1D in d2o (ref. to external acetone @ 31.07 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
MeS
13C
OH
NH2
180
160
140
120
100
80
60
40
20
0
ppm
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.23.u5_ob-07-191-c_loc3_10.35_C13_1D



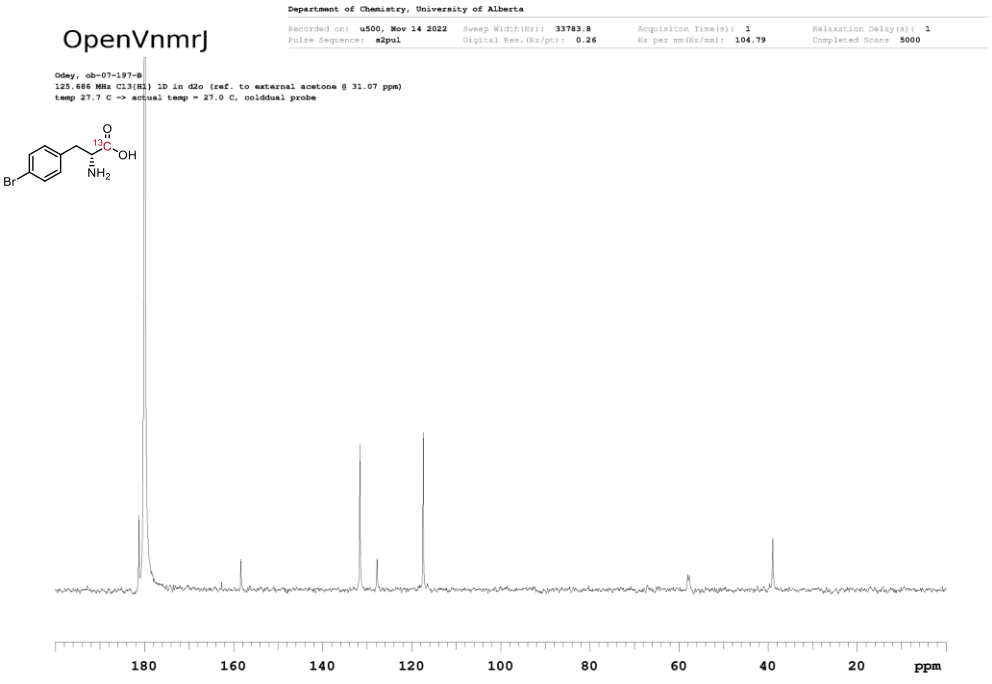
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.15.u5_ob-08-19-b-after_sec_col_locl_17.17_H1_1D



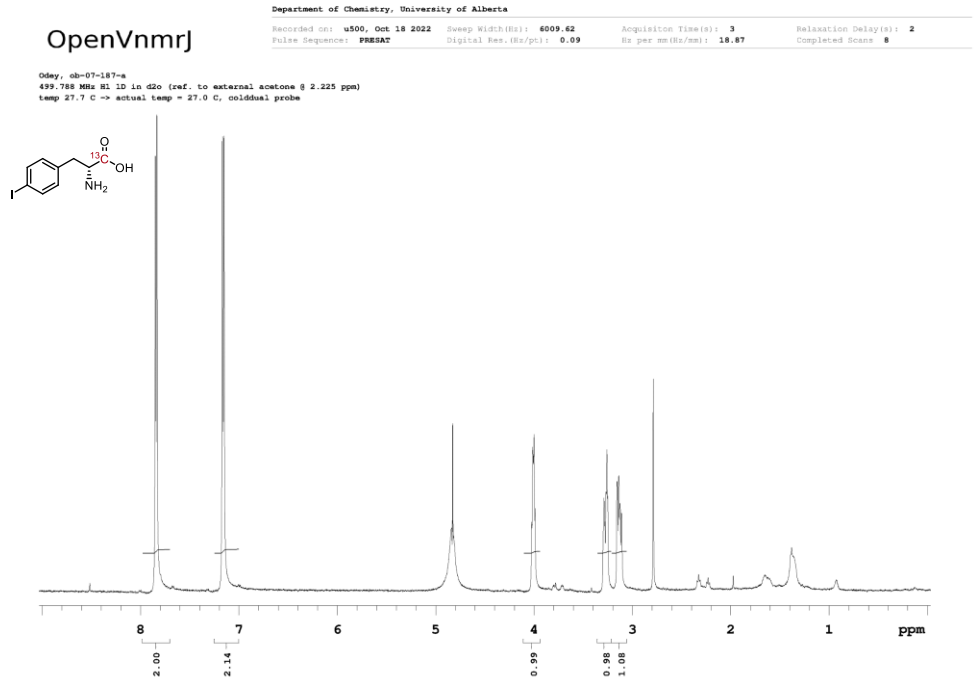
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.15.u5_ob-08-19-b-after_sec_col_locl_17.18_C13_1D

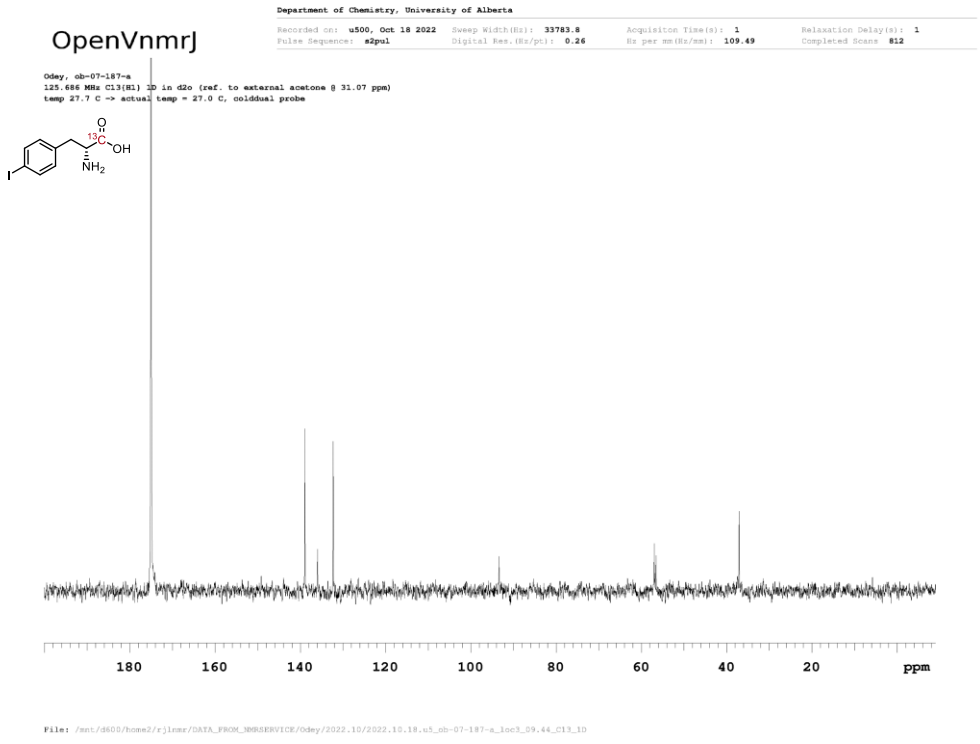


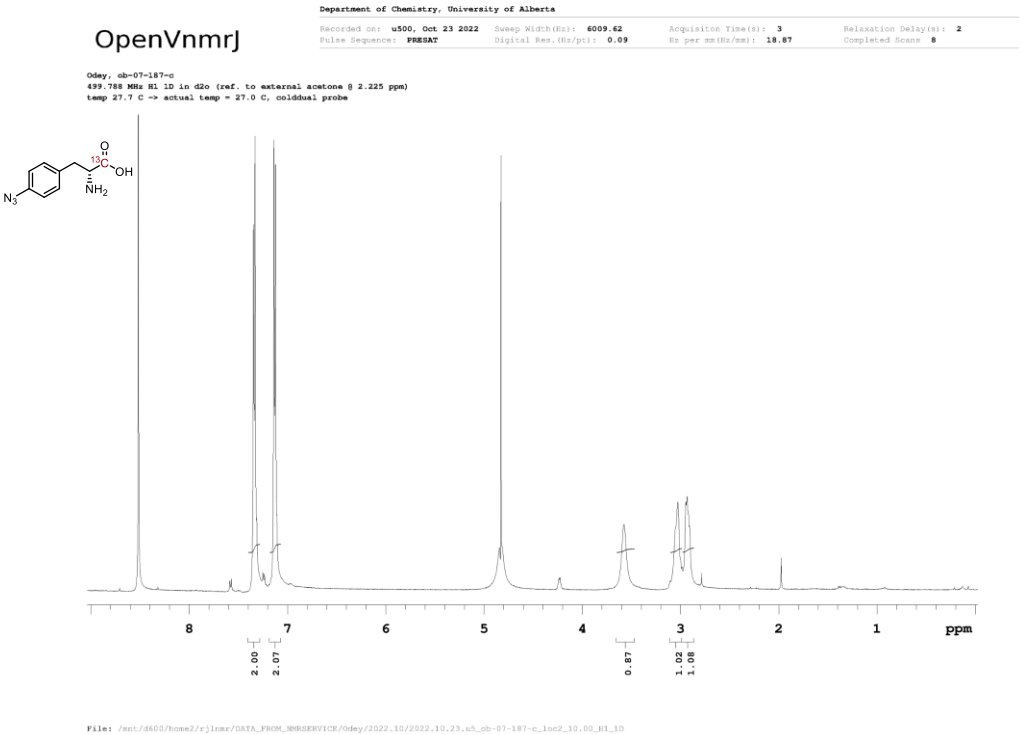
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.23.u5_ob-07-187-b-more_scans_loc5_12.22_H1_1D



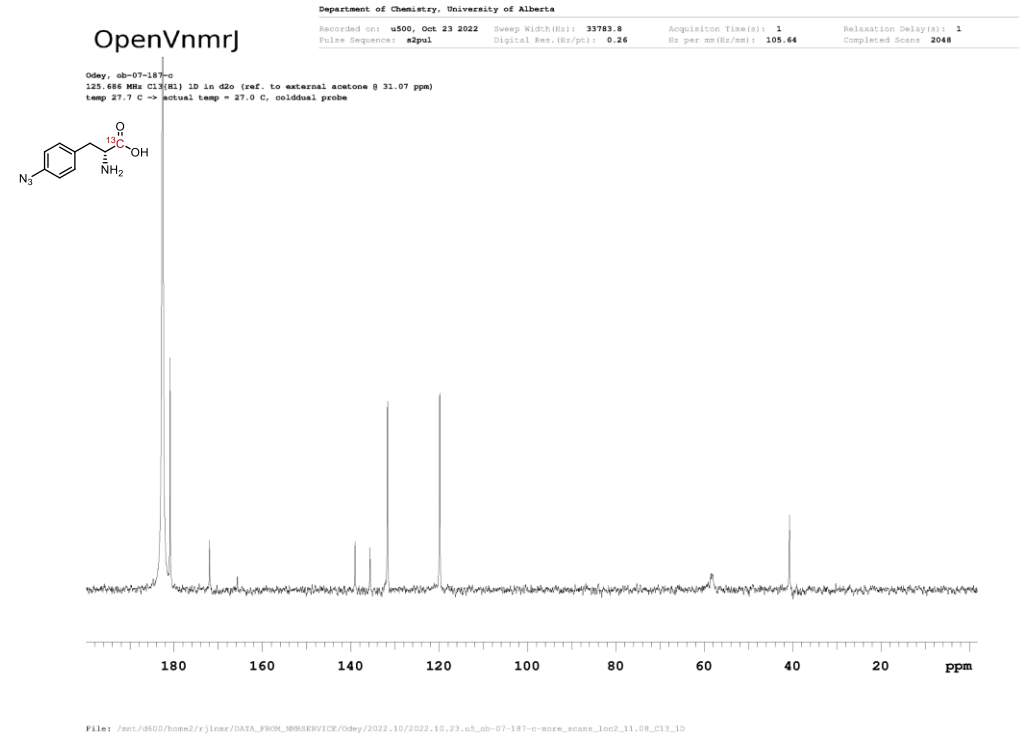
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.14.u5_ob-071-87-b-more_scans_loc6_20.05_C13_1D


OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: u500, Oct 18 2022 Sweep Width(Hz): 6009.62 Acquisiton Time(s): 3 Relaxation Delay(s): 2
Pulse Sequence: PRESAT Digital Res.(Hz/pt): 0.09 Hz per mm(Hz/mm): 18.87 Completed Scans 8
Odey, ob-07-187-a
499.788 MHz H1 1D in d2o (ref. to external acetone @ 2.225 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
O
13C
OH
NH2
I
8
7
6
5
4
3
2
1
ppm
2.00
2.14
0.99
0.98
1.08
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.18.u5_ob-07-187-a_loc3_10.13_H1_1D

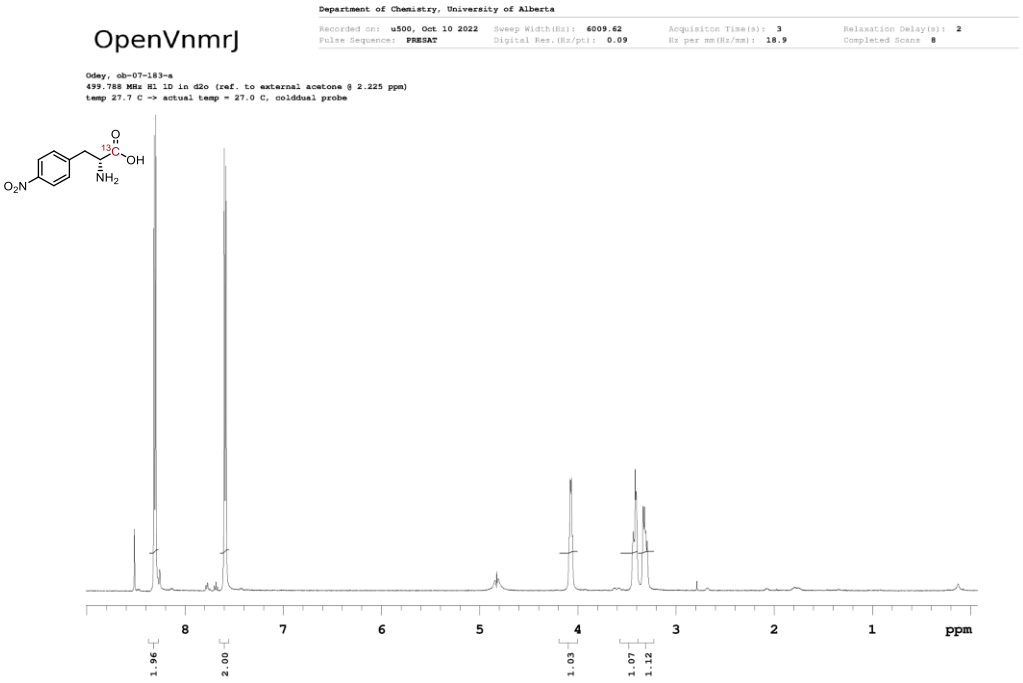

OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: u500, Oct 18 2022 Sweep Width(Hz): 33783.8 Acquisiton Time(s): 1 Relaxation Delay(s): 1
Pulse Sequence: s2pul Digital Res.(Hz/pt): 0.26 Hz per mm(Hz/mm): 109.49 Completed Scans 812
Odey, ob-07-187-a
125.686 MHz C13{H1} 1D in d2o (ref. to external acetone @ 31.07 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
O
13C
OH
NH2
I
180
160
140
120
100
80
60
40
20
ppm
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.18.u5_ob-07-187-a_loc3_09.44_C13_1D

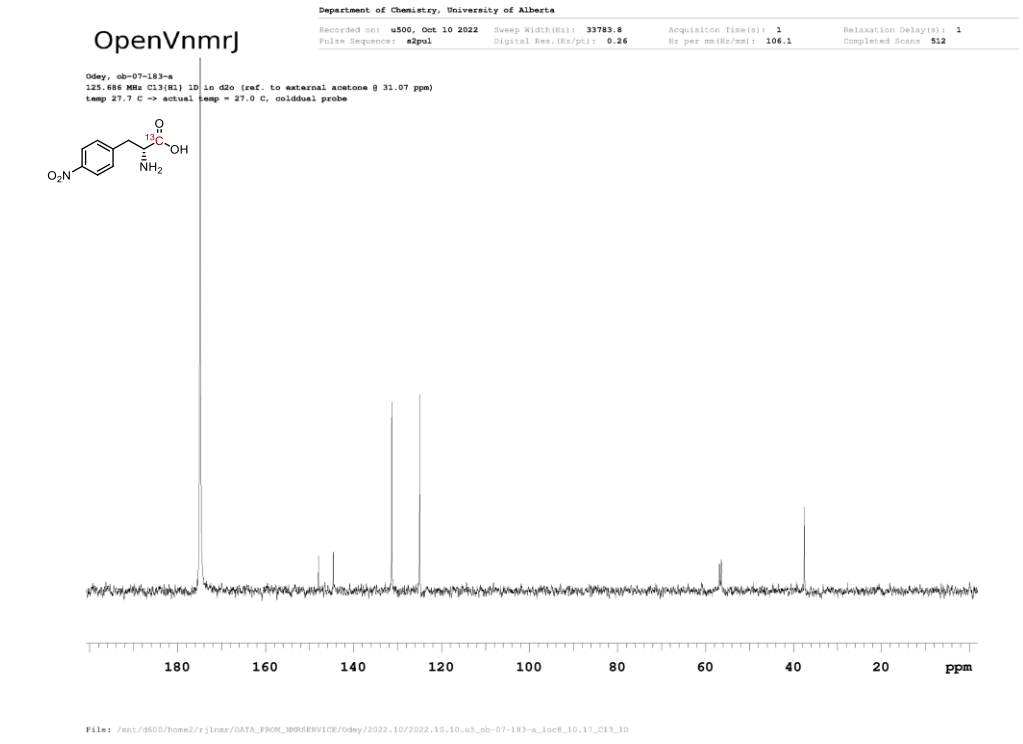


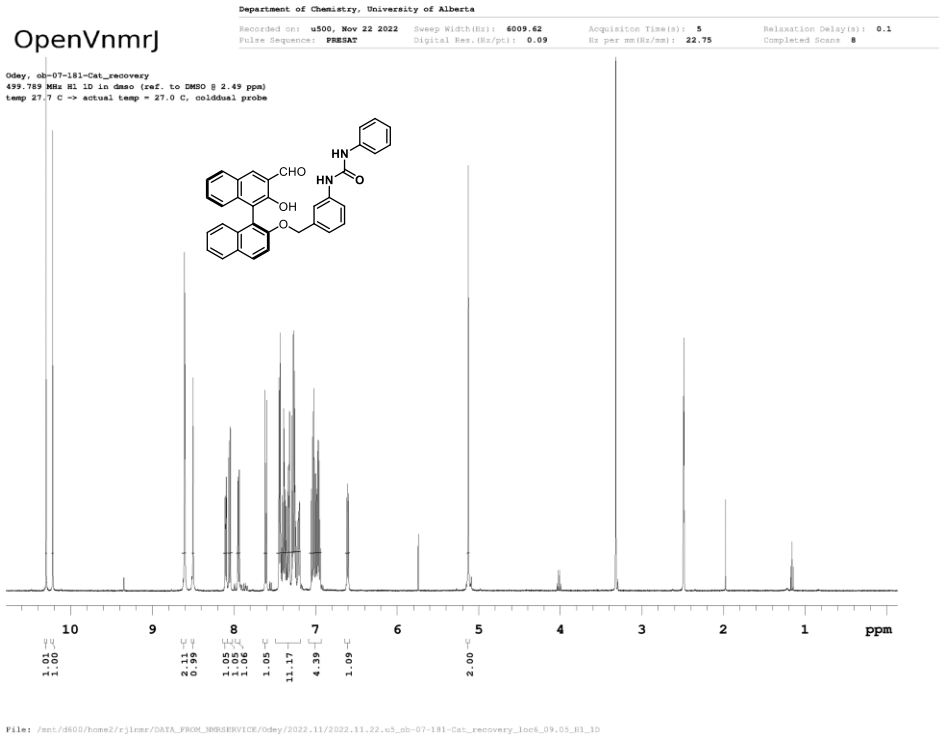
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.23.u5_ob-07-187-c_loc2_10.00_H1_1D



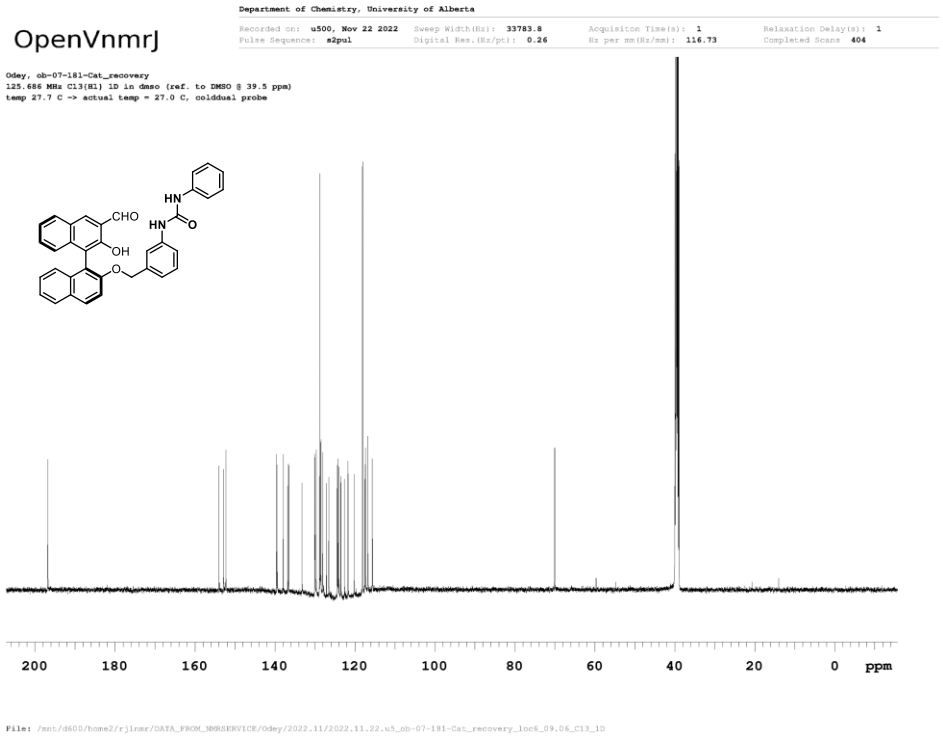
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.23.u5_ob-07-187-c-more_scans_loc2_11.08_C13_1D


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: u500, Oct 10 2022
Pulse Sequence: PRESAT
Sweep Width(Hz): 6009.62
Digital Res.(Hz/pt): 0.09
Acquisiton Time(s): 3
Hz per mm(Hz/mm): 18.9
Relaxation Delay(s): 2
Completed Scans 8
Odey, ob-07-183-a
499.788 MHz H1 1D in d2o (ref. to external acetone @ 2.225 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
O
13C
OH
NH2
O2N
8
7
6
5
4
3
2
1
ppm
1.96
2.00
1.03
1.07
1.12
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.10.u5_ob-07-183-a_loc8_10.17_H1_1D


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: u500, Oct 10 2022
Pulse Sequence: s2pul
Sweep Width(Hz): 33783.8
Digital Res.(Hz/pt): 0.26
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 106.1
Relaxation Delay(s): 1
Completed Scans 512
Odey, ob-07-183-a
125.686 MHz C13{H1} 1D in d2o (ref. to external acetone @ 31.07 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
O
13C
OH
NH2
O2N
180
160
140
120
100
80
60
40
20
ppm
File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.10/2022.10.10.u5_ob-07-183-a_loc8_10.17_C13_1D



File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.22.u5_ob-07-181-Cat_recovery_loc6_09.05_H1_1D



File: /mnt/d600/home2/rjlnmr/DATA_FROM_NMRSERVICE/Odey/2022.11/2022.11.22.u5_ob-07-181-Cat_recovery_loc6_09.06_C13_1D

CHAPTER 5

Conclusions and Future Work

5.1 Conclusions

Isotopically labeled drugs have attracted great attention in drug discovery programs due to their wide applicability and usage in all stages of drug development,[3-5] however, incorporation of an isotope into drug molecules remains difficult and time consuming. This thesis describes the development of reversible decarboxylation/carboxylation reactions of stabilized carboxylic acids and their derivatives to prepare isotopically-labeled carboxylic acids. Through careful pilot studies, reaction optimization, and mechanistic probes, the aryl aldehyde catalyzed carboxylate exchange of native α-amino acids with $*CO_2$ (* = 14, 13, 11) was discovered. Subsequent work revealed that a chiral aldehyde receptor can mediate the resolution/carboxylate exchange of native α-amino acids with $*CO_2$.

The work presented in Chapter 2 explored the direct reversible decarboxylation/carboxylation of stable organic acids in a polar aprotic solvent to enable incorporation of labeled $[^{13}C]CO_2$. We have utilized this finding to develop a new strategy for the isotopic labeling of electronically activated carboxylic acids with isotopically-labeled $[^{13}C]CO_2$, based on a reversible decarboxylation/carboxylation event of stabilized carboxylates. While there are many robust methods for the labeling of carboxylic acids with labeled carbon *C (* = 14, 13, 11), they often need either chemical activation-decarboxylation-metalation-carboxylation sequences mediated by transition metals,[112-113] indirect nucleophilic substitution reactions with labeled cyanide followed by hydrolysis,[76] or the introduction of labeled carbon monoxide in place of CO_2.[115-116] The work presented in Chapter 2 has enabled simple, direct protocols for isotopic exchange of carboxylic acids with $[^{13}C]CO_2$. Isotopically labeled carboxylic acids and their derivatives were prepared in high chemical and isotopic yield by simply supplying $[^{13}C]CO_2$ to carboxylate salts in polar aprotic solvents.

The work presented in Chapter 3 explored the carboxylate exchange of α-amino acids with $*CO_2$ (* = 14, 13, 11) catalyzed by aryl aldehydes to give access to C1-

labeled α-amino acids in a direct and operationally trivial manner. α-Amino acids are among the essential chemical building blocks of life.[136-139] Isotopically labeled α-amino acids and their derivatives have widespread use in structural and mechanistic biochemistry,[140] quantitative proteomics,[141] absorption distribution metabolism and excretion (ADME) profiling,[142-143] and as imaging agents in positron emission tomography (PET) techniques.[144-146] While there are robust methods to prepare of *C1-α-amino acids (* = 14, 13, 11), established methods involve label incorporation at an early stage of synthesis, are time consuming, and require multistep synthesis.[147-154] Our developed method granted access to C1-labeled products in a one pot strategy. Given the widespread use of labeled α-amino acids in discovery science, drug development, and medical imaging, we expect these findings to have immediate application. Productive catalysts must have balanced electrophilicity to enable carboxylate exchange without being irreversibly consumed in the process. Mechanistic studies indicate that the reaction likely proceeds via the trapping of $*CO_2$ by imine-carboxylate intermediates to generate aminomalonates that are prone to monodecarboxylation. The identification of the potential intermediate has allowed for the rapid and late-stage ^{11}C-radiolabeling of α-amino acids in the presence of $[^{11}C]CO_2$ through pre-generation of the imine carboxylate intermediate.

The work presented in Chapter 4 has explored the resolution/isotopic labeling of α-amino acids mediated by a urea-based binol aldehyde receptor in conjunction with $[^{13}C]CO_2$ via a reversible decarboxylation/carboxylation event of imine carboxylate intermediate. The method described in Chapter 3 provided racemic isotopically labeled α-amino acids; however, the proteins are consist of homochiral amino acids/peptides.

While there are some methods for the synthesis of enantioenriched isotopically-labeled α-amino acids with labeled carbon *C (* = 14, 13, 11), these methods involve the insertion of labeled carbon via classical methods such as cyanation/hydrolysis reactions using $[^{11}C]CN^-$,[156] and alkylation with $[^{11}C]CH_3I$,[157] which are followed by resolution methods. The resolution methods include enzymatic resolution,[147-149] the use of chiral auxiliaries for α-alkylation or α-amination,[154] and enantioselective hydrogenation of enamines.[155] To the best of our knowledge, the work presented in

Chapter 4 has explored the first example of resolution/carboxylate exchange of α-amino acids with $*CO_2$ (* = 14, 13, 11). This process is catalyzed by a chiral urea-based binol aldehyde receptor to give general access to C1- enantioenriched labeled α-amino acids in one pot strategy.

5.2 Future Work

The work described in this thesis provides a foundation for isotopic labeling of carboxylic acids and α-amino acids with labeled carbon $*CO_2$ (* = 14, 13, 11). The most promising reaction platform for the expansion of these methodologies is to exploit isotopic labeling in the context of asymmetric catalysis further.

The resolution/carboxylate exchange of α-amino acids with $*CO_2$ (* = 14, 13, 11), catalyzed by a chiral urea-based BINOL aldehyde receptor, as described in Chapter 4, includes the report of optimized reaction condition, the scope of most proteinogenic α-amino acids, and some non-natural variants containing potentially reactive functional groups. Other necessary understanding and expansion of the scope to include more non-natural variants of the process are yet to be explored fully. The process is thought to be similar to the carboxylate exchange of α-amino acids with $*CO_2$ (* = 14, 13, 11), catalyzed by achiral aryl aldehydes to give general access to the C1-labeled products, described in Chapter 3. The stereoselectivity observed requires further kinetic and mechanistic experiments for efficient understanding of this new process.

The work described in Chapter 4 on the resolution/carboxylate exchange of α-amino acids with $*CO_2$ (* = 14, 13,11) provides access to enantioenriched isotopically-labeled α-amino acids in one single step. Although a good enantiomeric ratio was achieved in this process, the development of a new chiral aldehyde that can lead to enantioselective isotopically-labeled α-amino acids would be the ideal methodology (**Fig. 5–1a**). Another approach to access isotopically-labeled homochiral α-amino acids would be to use the chiral aldehyde described in Chapter 4, followed by adding D-amino acid oxidases (DAAO) and catalase in situ, which will eliminate the need of purifying the product after the labeling step, then subjecting it to the enzymatic oxidation conditions by DAAO (**Fig. 5–1b**).

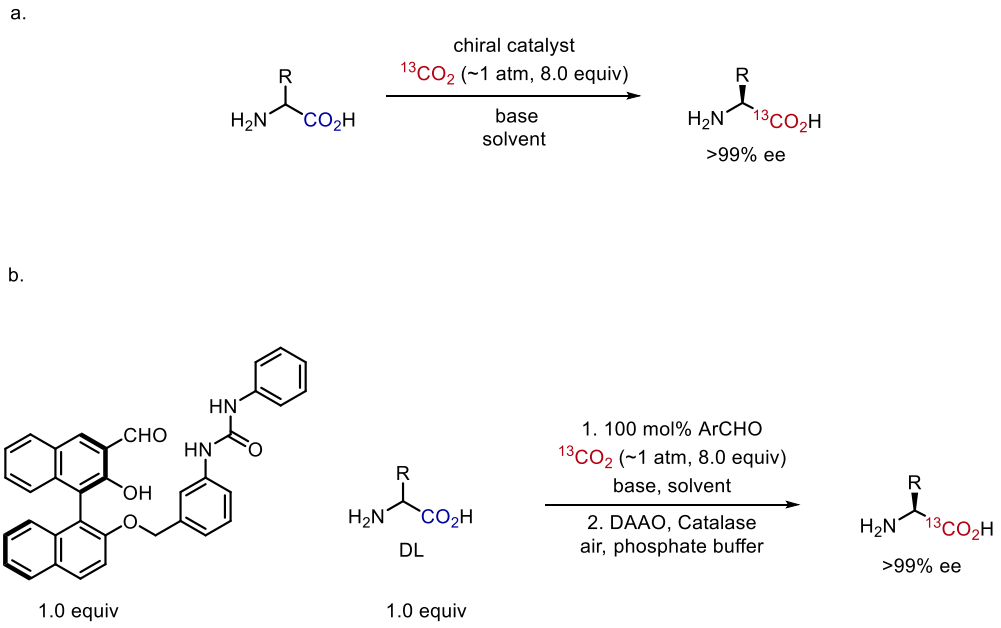


Fig. 5–1. Future directions to access enantiomerically pure isotopically labeled α-amino acids.

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